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(From the portrait by Deane Keller)

SOME ENDOCRINE INFLUENCES ON SKELETAL GROWTH AND DIFFERENTIATION*

MIRIAM E. SIMPSON, C. WILLET ASLING, AND HERBERT M. EVANS

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Introduction

In the skeleton, growth is measured by increase in size and particularly by increase in length of the axial and appendicular skeleton, whereas differentiation or maturation is measured by the establishment of ossification centers and their subsequent fusion. In normal development these processes of increase in size and differentiation proceed concurrently. The smoothly coordinated mechanism may, however, become deranged. In man dwarfism may occur, although the open epiphyses would indicate further capacity for growth, yet continued open epiphyses may also be associated with gigantism. An understanding of the hormones controlling skeletal growth therefore necessitates an analysis of the factors, or balance of factors, responsible for each of these phases of growth—increase in size, and differentiation.

As dwarfism results from removal of the pituitary, attention is immediately focused on the role of the hormones of the pituitary and of its target organs. Dwarfism and retarded skeletal maturation also accompany thyroid deficiency; it is therefore evident that among the target organs the thyroid must receive prominent attention. One pituitary hormone, the growth

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hormone, is unique in that its effects are demonstrable in the absence of all the target organs.

Requirements for skeletal studies

Techniques used. Increase in size of the skeleton may be determined by direct measurements of the total length of the animal, usually under anaesthesia or at autopsy. Skeletal growth of small experimental animals, such as the rat, can also be followed at suitable intervals during life, and without anaesthesia, by direct measurement of the tail. The size of individual bones can be determined by measurement after dissection, or in roentgenograms. These methods of measuring skeletal growth in the rat have a maximal error of 2%.

In the study of differentiation of the skeleton, roentgenograms are of great service in tracing both the appearance and disappearance of centers of ossification. Confirmation of the accuracy of roentgenographic determinations has been derived from parallel histological studies, particularly in the determination of epiphyseal fusion.*

Histological sections also allow analysis of the state of activity, i.e., the processes which lead to the formation of the epiphyseal disc and its removal. Briefly, some of these characteristics are: number, size and vacuolation of cartilage cells, orderliness of alignment, amount and quality of matrix, number of capillaries impinging upon cartilage, the number and size of osteoblasts and osteoclasts, also the number, size, and arrangement of primary and secondary bony trabeculae.

The rat as an experimental animal. As some question has been raised as to the suitability of the rat as an experimental animal in studies of skeletal growth, a defense of the use of this animal will be made. A common opinion (as expressed by Washburn[71]) is that inasmuch as the rat does not show maturity by epiphyseal closure and is hence capable of continued growth far past the period of sexual maturity, deductions of general import cannot be made from the reactions of its skeleton. The position taken here is that in spite of certain peculiarities in the growth of the skeleton, the rat is useful in a study of the hormonal factors controlling skeletal growth. As a matter of fact, the majority of epiphyses in the rat do close, and the times of closure of these epiphyses are accurately known. Further, most of them close in early adult life (80 to 120 days). For example, all epiphyses of the paws close and, in addition, at least one of the two epiphyses possessed by the other long bones of the extremities. (The ulna and femur are exceptions.) The epiphysis of the metacarpal closing at 110 days exemplifies this group.[14]

Those epiphyses which remain open are in the minority. They occur notably in the limbs and tail. Each of the major long bones has one epiphysis which remains open into senescence. In the ulna and the femur both epiphyseal discs remain. Patency of the epiphyses of caudal vertebrae can also be recognized histologically even in old age.

---

* For accuracy in analysis of roentgenograms of bones of small animals a fine grain emulsion film is essential; for accuracy in histological deduction greatest care must be taken in determining the plane of section.

The proximal epiphysis of the tibia is one of the carefully studied epiphyses of the type which does not close until late in life. Even though such an epiphyseal cartilage remains, the conclusion is unwarranted that continued growth is occurring in this bone. Abrupt narrowing of the plate has been noted in early adult life at the time of closure of the majority of epiphyses. Such persisting epiphyseal discs become dormant, sealed by bony plates from the marrow. The growth curve of the tibia (Fig. 1) shows that its length does not increase over long periods although the epiphysis remains patent into senility. It will be noted that the leveling off of the growth curve coincides with the abrupt narrowing of the epiphyseal disc.

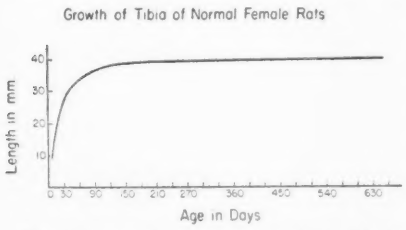

FIG. 1. Curve showing growth of the tibia in normal female rats of the Long-Evans strain.

Determination of skeletal age. The epiphyseal closures around the 110th day of life occur with such reliability within a limited period characteristic for each bone that they can be used as an index of skeletal ageing. By comparison of the skeletal differentiation in an experimental animal with that in normal rats of known chronological ages, it is possible to assign the "skeletal age." For determination of skeletal ages at less than 80 days, the time of appearance of new epiphyseal ossification centers is more important than the time of epiphyseal closure. Table 1 shows the times of appearance of centers which are important in determining skeletal ages in the period between 12 and 60 days of age. It will be noted that this table is devoted exclusively to times of appearance and disappearance of the secondary ossification centers, all primary centers having been established in the rat by birth or shortly thereafter.*

Although the closure of some epiphyses is greatly delayed in the rat, the sequence of closure follows the general mammalian pattern (Todd, as cited by Krogman[38]). In Table 2 the order of closure in rat and man is compared.

In order to use the rat in experimental studies on osteogenesis it is only necessary to know the periods during which secondary centers appear or

* Additional criteria are available for the earlier group (12 to 60 days) and appear in the publications from which the table has been adapted (Scow *et al.*[55]; Ray *et al.*[48]). These criteria are chiefly determined from roentgenograms, though histological confirmation has been obtained for the proximal tibia, metacarpals, and distal humerus (Becks *et al.*[9, 12, 14]). The criteria for determination of skeletal ages between 80 and 120 days are chiefly histological. It will be noted that no satisfactory criteria are presented for skeletal ageing between 60 and 80 days, or between 120 and 940 days.

TABLE 1

DETERMINATION OF SKELETAL AGES

Ossification center	*Chronological age in days*														
	12	15	18	21	25	30	40	50	60	80	100	120	Latent period	940	1006-1091	1135+
Humerus, proximal	P	—	—	—	—	—	—	—	—	—	—	—	.....	—	—	A
distal	P	—	—	—	—	—	—	A								
medial epicondyle				P	—	—	—	—	—	—	—	A				
Radius, proximal		P	—	—	—	—	—	—	—	—	A					
distal	P	—	—	—	—	—	—	—	—	—	—	—	.....	—	—	A
Ulna, proximal		P	—	—	—	—	—	—	—	—	—	—	.....	—	A	
distal	P	—	—	—	—	—	—	—	—	—	—	—	.....	—	—	A
Metacarpal, distal	P	—	—	—	—	—	—	—	—	—	A					
Phalanges		PI	—	PII	—	—	—	—	—	—	A					
row I, II, III				PIII												
Femur, proximal								P	—	—	—	—	.....	—	A	
trochanter						P	—	—	—	—	—	—	.....	—	A	
distal	P	—	—	—	—	—	—	—	—	—	—	—	.....	—	—	A
Patella						P										
Tibia, proximal	P	—	—	—	—	—	—	—	—	—	—	—	.....	—	—	A
distal	P	—	—	—	—	—	—	—	—	—	A					
Metatarsal, distal	P3	P2	P4	P1	—	—	—	—	—	—	A					
			P5													
Pelvis, acetabulum							P	—	A							
lip																
triradiate								A								

"P" represents present; "A" represents absent.
Adapted from Scow *et al.*,[55] Ray *et al.*,[48] and Dawson.[19]

TABLE 2

SEQUENCE OF EPIPHYSEAL CLOSURE

*Rat** *Age in days*	*Epiphyses*	*Man†* *Age in years*
40	Humerus, distal (capitulum-trochlea)	14-15
85	Radius, proximal	16-17
90	Tibia, distal	16-17
100	Metacarpal, distal	16-17
120	Humerus, distal (medial epicondyle)	16-17
1135	Tibia, proximal	17-18
1135	Femur, distal	17-18
1135+	Radius, distal	18-19
1135-1270	Humerus, proximal	19-20

* Histological studies of the authors supplemented in later chronological ages by data from Dawson.[19]
† Todd (1933),[64] modified by Krogman (1941).[30]

become fused.* During these critical periods experimental contrivances which significantly hasten or retard differentiation should be as significant as when applied to other adequately studied mammalian forms.

Dietary and environmental factors. In studies of the effects of hormones on skeletal growth particular care must be taken to control dietary and other environmental conditions. The diets must be adequate in amount and complete in all known components. It has been shown that dietary restriction can cause changes in the skeleton similar to hypophysectomy. In starved animals bone growth ceases, epiphyseal plates are narrower and less active than normal.[7, 32] In experiments where the hormone administered affects the appetite, it may be necessary to do paired-feeding in order to get significant results. Even in the presence of adequate caloric intake, vitamin deficiencies can cause similar effects.[27] Diminished food consumption such as occurs after hypophysectomy may lead to deficient vitamin intake. On the other hand, vitamin requirements may be increased by hormone therapy. Thyroxin increases the need for practically all vitamins.[22] This is especially noticeable in the B vitamins.[20]

The salt content of the diet must be adequate, since it is known that mineral deficiency (e.g., magnesium deficiency) will produce effects with some resemblances to hypophysectomy.[4]

The amount of protein in the diet is also significant. Low protein diets will produce changes in bone not unlike hypophysectomy (Nelson, Sulon, and Becks, unpublished). It has been claimed repeatedly that high protein diets will promote growth in excess of normal, but in many of these cases the stock diet with which comparisons were made were low in protein.[45]

Forced feeding has been reported to lead to growth of hypophysectomized rats.[40, 52] Such increase in weight is, however, largely due to accumulation of fat; true skeletal growth has not been proven.

In all studies on bone growth from the Institute of Experimental Biology which are reported here greatest care has been taken to maintain a satisfactory environment and to supply an adequate diet. The hypophysectomized rats, which form the basis of much of the experimental work, have been given a modified MacCollum's diet (Diet I). (It is true that very old hypophysectomized rats maintained on this diet do accumulate some fat so that the body weight increases slowly in old age; however, this weight gain does not indicate growth.) Survival of hypophysectomized animals, under the favorable conditions specified, is adequate for chronic experimental procedures; considerable numbers have been maintained for more than two years, some for three-year periods.

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* A useful summary of the roentgenographic and histological standards for the rat will soon be published in atlas form by Becks *et al.*

Effects of endocrine gland ablations

Effects of hypophysectomy. The results of hypophysectomy on skeletal growth will first be described for a rat of an age and sex commonly used in experimental studies, the 28-day female. Virtually all primary and secondary centers of ossification are present at this age. After the operation, such an animal ceases growth almost at once, both in weight and length. The changes in the osseous system will be described first as seen in the tibia, a bone which has been used so commonly in testing effects of experimental procedures. This bone normally retains an epiphyseal disc at its proximal end until late in life. Following hypophysectomy, the epiphyseal disc soon shows evidence of inactivity. Its width decreases and by two weeks it is far below normal. The cartilage cells of the disc decrease in size; the vacuolated juxta-medullary zone disappears; osteoblasts decrease in size and lose their epithelioid character. Fine trabecular bone of the primary spongiosa disappears and only a few coarse secondary trabeculae remain. The epiphyseal cartilage plate finally becomes sealed from the marrow by a lamina of bone, but persists even into old age. As a result of this premature arrest of growth, the plate resembles that of the senescent normal rat. Other epiphyses, which, like the tibia, do not close until late in life, likewise become sealed and persist.

It is interesting that the sealing lamina of bone found in the rat after hypophysectomy resembles closely the condition described by Erdheim[21] in a case of human pituitary dwarfism (Figs. 2-3).

Epiphyseal plates which normally disappear between 80 and 100 days of age (proximal radius, distal tibia, the metacarpals, and the medial epicondyle of the humerus) also fail to fuse after hypophysectomy. They become sealed by bone and have been observed to persist for one to two years. The failure of such epiphyses to close in rats hypophysectomized at 28 days is evidence that deprival of pituitary hormones gravely disturbs normal osseous differentiation. (This effect of hypophysectomy in delaying epiphyseal closure was first shown by Dandy and Reichert, in 1938, by roentgenographic study of hypophysectomized dogs.[18])

The epiphysis at the distal end of the humerus which normally fuses between 31 and 42 days of age continues differentiation and closes after hypophysectomy at 28 days of age. Therefore hypophysectomy does not immediately arrest the normal progress of differentiation, and epiphyseal centers whose fusion is imminent progress to closure. After hypophysectomy at 28 days of age the advance in skeletal differentiation leads to a bone age of 47 days. The effect of hypophysectomy at 28 days on epiphyseal closure is summarized in Table 3.

If it is a general principle that hypophysectomy delays closure of epiphyses, it should be possible by earlier hypophysectomy so to check skeletal differentiation that the earliest closing epiphyseal plates would

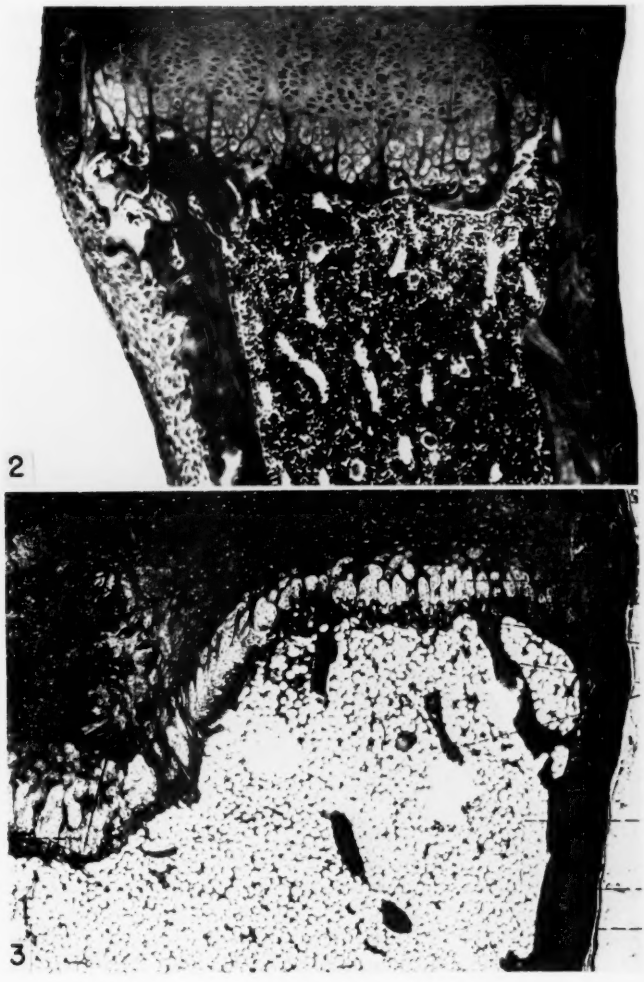

PLATE I

Comparison of experimental hypophysectomy with a case of human pituitary dwarfism.

FIG. 2. Costochondral junction of hypophysectomized 60-day-old female rat, 39 days postoperative. (H & E stain, x106.) (After Ray *et al.*[42])

FIG. 3. Costochondral junction from human male, 38 years of age; diagnosis nanosomia pituitaria. (After Erdheim.[21])

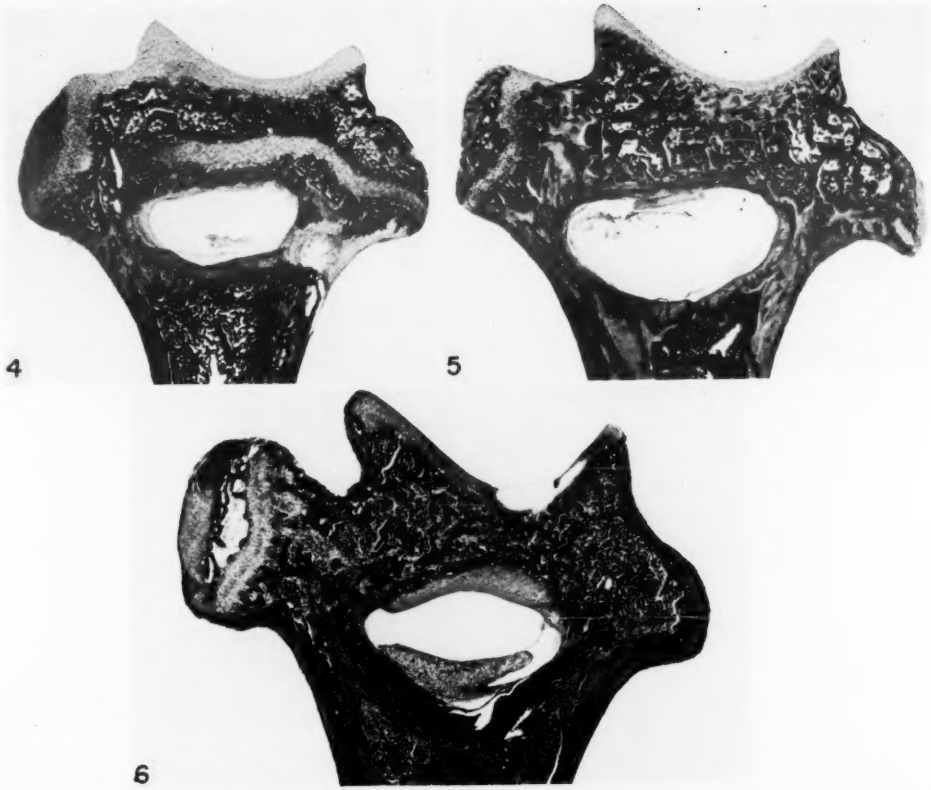

PLATE II

remain intact, for instance that in the distal humerus. It was found that if hypophysectomy was performed at 13 days,[3, 70] arrest of differentiation occurred much sooner and only partial closure occurred in the distal humerus. In assigning to these rats a skeletal age of 33 days, complete dependence was not placed on epiphyseal closure. The appearance of new secondary centers was even more helpful. (By again consulting Table 1, showing the differentiation of the skeleton at progressive chronological

TABLE 3

EPIPHYSEAL STATUS OF RATS HYPOPHYSECTOMIZED AT 28 DAYS OF AGE AFTER A POSTOPERATIVE PERIOD OF 480 DAYS

Epiphyseal center	*Normal rats* *Age of epiphyseal closure in days*	*Hypophysectomized rats* *Epiphyseal status*
Humerus, distal	40	—
Radius, proximal	85	+
Tibia, distal	90	+
Metacarpal, third	100	+
Humerus, medial epicondyle	120	+
Ulna, olecranon	940	+
Humerus, proximal	1135	+
Radius, distal	1135	+
Ulna, distal	1135	+
Fibula, proximal	1135	+
Tibia, proximal	1135	+

Modified from Evans *et al.*[77]
Epiphyses separate are marked +; those fused are marked —.

ages, it will be seen how many secondary centers appear during this early period, 13-33 days). Rats hypophysectomized at day 6 showed an advance in skeletal age to 24 days. The operation at this very early age delayed markedly both the fusion of established centers of ossification and

PLATE II

Effects of hypophysectomy at different ages on the degree of differentiation attained by the humerus of the rat at 60 days of age. (H & E stain, x14.)

FIG. 4. Rat hypophysectomized at 6 days of age. The main epiphyseal plate has persisted. Establishment of the medial epicondylar ossification center has not occurred. Bone age 20 days.

FIG. 5. Rat hypophysectomized at 28 days of age. Epiphyseo-diaphyseal union at the main plate has occurred in spite of the operation. The plate in the medial epicondyle remains. Bone age 40 days.

FIG. 6. Normal 60-day-old rat. The main epiphyseal plate has disappeared at the normal time (40 days), leaving only that in the medial epicondyle. (After Asling *et al.*[3])

the acquirement of new secondary centers. All centers present were found to be sealed from marrow by bone. The much greater retardation in skeletal differentiation occasioned by hypophysectomy at day 6 than at day 28 is illustrated in Figures 4-6 which show the degrees of development attained at the distal end of the humerus.

Table 4 also shows the extent of differentiation of the skeleton which had taken place by the time a chronological age of 60 days was reached following hypophysectomy at progressively earlier ages. Further, it will be noted here that the advance in skeletal age was constant, being 18 to 21 days, regardless of whether the hypophysectomy was performed at 28, 21, 13, or 6 days of age.* It appears then that there is no more potentiality for skeletal differentiation independent of the pituitary at earlier ages than at older ages.

TABLE 4

ESTIMATED SKELETAL AGE IN DAYS AT 60 DAYS CHRONOLOGICAL AGE FOLLOWING HYPOPHYSECTOMY AT PROGRESSIVELY YOUNGER AGES

Age at hypophysectomy	*Skeletal age at 60 days*	*Advance in skeletal age*
28	47	19
21	42	21
13	33	20
6	24	18

Adapted from Asling *et al.*[3]

On the other hand increase in body size (weight and length) following hypophysectomy at these progressively younger ages was arrested at a constant chronological age, 30 days. As the daily increment was approximately the same in all groups, the total weight and length gain between the day of operation and 30 days chronological age was greater in the younger of these groups (Figs. 7 and 8). It appears, therefore, that whereas maturation continues for a constant interval after operation, 20 days, increase in size persists to a fixed set chronological age (30 days). The evidence from hypophysectomy thus indicates that skeletal differentiation is under a control different from increase in size.

In seeking an explanation for the persistence of osseous differentiation during the definite period after hypophysectomy, attention was directed to

* It may be possible to show that this constancy of the progress in differentiation following hypophysectomy applies to animals older than those cited, provided hypophysectomy is performed in periods where adequate criteria are available for determination of skeletal age. Asling *et al.*[3] have noted progress for 20 days in the differentiation of the metacarpal in rats hypophysectomized at 75 days.

the thyroid hormone due to the well-known effect of this hormone on skeletal maturation. This hormone is known to continue to give effects for a considerable period after administration.[33] An effect of thyroid hormone on differentiation after hypophysectomy could be due to continued low-grade activity of the thyroid, or to persisting action of thyroid hormone already present in the body at the time of operation. (The persistence of

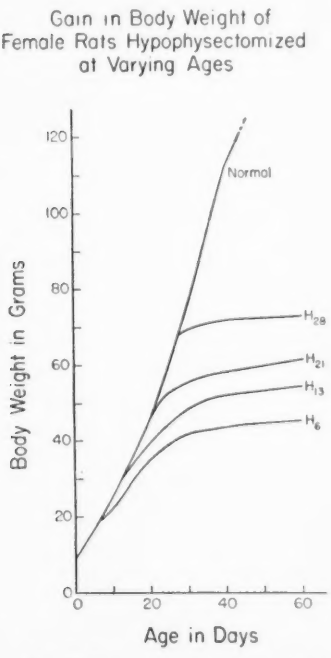

FIG. 7. Curves showing gain in body weight of rats hypophysectomized at 6, 13, 21, or 28 days of age. (After Asling *et al.*[3])

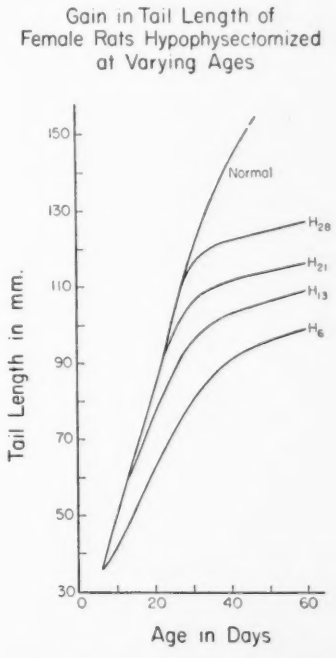

FIG. 8. Curves showing increase in length of the tail of rats hypophysectomized at 6, 13, 21, or 28 days of age. (After Asling *et al.*[3])

action of pituitary hormones, especially growth hormone, would not be probable, as pituitary hormones are characterized by short survival of their effects (Van Dyke *et al.*, unpublished).

Effects of thyroidectomy. In considering the role of the thyroid hormone in osseous growth and differentiation, the effects of thyroid ablation will first be described. As in the case of pituitary deprivation, it was desirable to study the effects of thyroidectomy at as early an age as possible. Salmon[50, 51] had shown that thyroidectomy could be performed on the first day of life, and through the collaboration of Scow[53, 54, 55] and Ray[46, 47] we have

been able to study the effect of thyroid deprival at this early age.* It was found that skeletal age advanced to 18 days by a chronological age of 60 days. The differentiation undertaken by thyroidectomized rats was then of the same order as was achieved by hypophysectomized rats by the same chronological age, though the actual skeletal age attained was less, as the thyroidectomy was performed earlier than the earliest hypophysectomy.

Although growth was markedly retarded and the skeletons of thyroidectomized rats resembled those of hypophysectomized rats in their small size, and in the unexpanded epiphyseal centers, the bones differed in several important respects. The epiphyseal cartilages did not become sealed by bony plates, either on the epiphyseal or diaphyseal sides, even after prolonged postoperative periods.†

Some degree of proliferative activity persisted in the cartilage plates, and some erosion was still occurring. However, the plate was not reduced in width normally with increasing chronological age; by 56 days of age the plates were found to be actually wider than normal. This condition contrasts with that in hypophysectomized rats where the plate shows almost no activity, is reduced in width at once, and where no erosion can occur as the cartilage is sealed from marrow by bone.

When the thyroidectomized rats were allowed to survive for long periods, some very slow continued differentiation of the skeleton was detected. Scow and Simpson[68] noted that in animals which survived for 120

* The parathyroids were removed with the thyroid. Rats are known to be very resistant to parathyroidectomy, rarely developing tetany, especially if on a high calcium diet. As these thyro-parathyroidectomized rats were on a diet high in calcium and as tetany was observed only in a few instances, and then only transiently, and as chemical analysis showed complete mineralization of the bones of such animals when placed under the influence of thyroxin, it was considered justifiable to omit consideration of the parathyroids for the present.

† Becks *et al.*[5] had noted in rats thyroidectomized at somewhat greater age that sealing of cartilage plates by bone did not occur; persisting unsealed plates were observed in the proximal tibia 17 months after operation. In the rats thyroidectomized at day 1, persisting unsealed plates have been observed in the tibia, caudal vertebrae, and metacarpal.

PLATE III

Comparison of the effects of thyroidectomy and of hypophysectomy on the humerus of the rat. All specimens 60 days of age. (H & E stain, x14.)

FIG. 9. Thyroidectomized day 1. The main epiphyseal ossification center has appeared but is unexpanded. There is no ossification center in the medial epicondyle.

FIG. 10. Hypophysectomized day 20. The main epiphyseal cartilage plate has been removed except for a few fragments in the lateral epicondyle. The medial epicondylar ossification center and its cartilage plate are well established.

FIG. 11. Thyroidectomized day 1, subsequently hypophysectomized day 20. Only the main epiphyseal ossification center has appeared. Note that in the absence of the thyroid the advance of ossification which follows hypophysectomy has not occurred.

FIG. 12. Normal control of the same chronological age. Complete fusion has occurred at the main epiphyseal plate. Note the active epiphyseal plate in the medial epicondyle and the resulting prominence of the epicondyle.

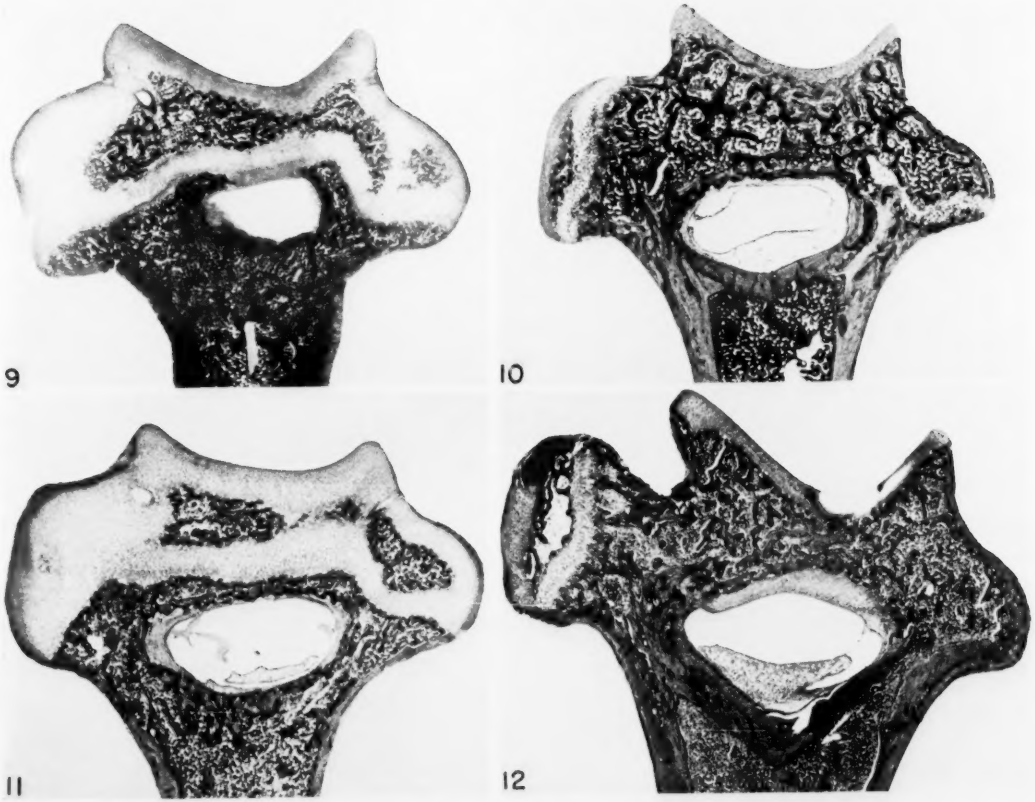

PLATE III

to 140 days, the bone age had advanced to 20 or 24 days.* It will be remembered that in the hypophysectomized rats differentiation ceased completely after a three-week advance; no further differentiation occurred even though the animals survived for over a year.

The bones of the thyroidectomized rats also differed from those of hypophysectomized rats in that the primary trabeculae remained delicate. In hypophysectomized rats the fine trabeculae were soon transformed, and only a few coarse trabeculae remained continuous with the sealing lamina of bone.

TABLE 5

COMPARISON OF THE EFFECTS OF THYROIDECTOMY AND HYPOPHYSECTOMY ON SKELETAL MATURATION IN THE RAT

Operation	*Age at operation* Days	*Skeletal age at 60 days* Days	*Observer*	*Advance in skeletal age after operation* Days
	1	15	Ziskin *et al.* (1940)	14
	1	18	Scow *et al.* (1948)	17
Thyroidectomy	1	23	Ray *et al.* (1950)	22
	7	23	Ziskin *et al.* (1940)	16
	6	24	Walker *et al.* (1950)	18
	13	33	Walker *et al.* (1950)	20
Hypophysectomy	21	42	Ray *et al.* (1950)	21
	28	47	Walker *et al.* (1950)	19
	75	95	Asling *et al.* (1949)	20
Thyroidectomy and hypophysectomy	1 20	18	Walker *et al.* (unpubl.)	17

Since slow growth as well as slow differentiation characterizes the thyroidectomized rat, some continued pituitary function, such as the secretion of small amounts of growth hormone, might be assumed. It is known that the pituitary is badly deranged after thyroidectomy; the alpha cells (which are supposed to produce growth hormone) are recognizable in only very small numbers, but it is possible that some activity persists in them. Although this might account for the growth, it is improbable, as will be seen later, that growth hormone would influence the slow matur-

* The differentiation in the skeleton of the thyroidectomized rats was already very slow by 30 days of age. At this time a bone age of 15 days had been attained and the bone age only advanced 3 days in the ensuing 30 days.

ation. If one assumes that the slow but sustained skeletal growth following thyroidectomy is due to continued production of small amounts of growth hormone, and that the limited but definite advance in skeletal differentiation following hypophysectomy is due to continued action of thyroxin, or to low-grade thyroid activity, then simultaneous removal of the two organs should give more complete stasis than either operation alone. The earlier the two operations could be performed, the more striking the retardation should be.

Effects of combined thyroidectomy and hypophysectomy. It has been possible[70] to combine thyroidectomy on day 1 with hypophysectomy on day 20. It was found that the bones of the doubly operated animals resembled those of thyroidectomized rats in that the cartilage plates were not sealed by bone. The skeletal age was the same as after thyroidectomy alone, a skeletal age of 18 days being attained by 60 days. A comparison at a chronological age of 60 days of the skeletal ages of hypophysectomized, thyroidectomized, and hypophysectomized-thyroidectomized rats is shown in Table 5. The estimates of skeletal age of several workers who have studied the effects of one or more of these operations are included, and it is interesting to note the close agreement in data obtained.

The histological findings in rats subjected to the three types of operations may be compared from sections of the humerus (Figs. 9-12).

Effects of hormone administration on skeletal development— Growth hormone

The pituitary growth hormone has been tested for its capacity to restore growth and cause differentiation in the skeletal system of deficient rats (hypophysectomized, thyroidectomized, and doubly operated animals) and has been tested for its effects when given in excess to normal rats.

Growth hormone in normal rats. When normal adult female rats which have reached the growth "plateau" are injected with growth hormone, growth in weight and length is resumed and gigantism eventually results. All ossification centers present respond. It is to be remembered that only epiphyseal plates belonging to the group characterized by greatly delayed closure are still present in such mature rats.

If, however, growth hormone administration is begun at earlier ages, great differences become evident in the responsiveness of different parts of the skeletal system. These variations in response seem to be related to the periods of closure of epiphyses characterizing individual bones. In general it may be said that all epiphyses respond to growth hormone unless at the onset of injection the epiphyses are too near the time of normal closure. For example, in animals in which injections were begun at 81 days of age the epiphysis of the metacarpal continued differentiation and closed at the normal time (110 days) without elongation of the bone.[1]

This was equally true of the distal epiphysis of the tibia (Asling *et al.*, in press*). In the same animals the proximal tibial epiphysis responded and in the 30-day injection period the tibia achieved its definite length, that usually attained by 250 days. Even after chronic administration, when the tibia had exceeded normal limits by 15%, its epiphysis still remained open and capable of response.[27] The evidence is, therefore, that the response of different bones of the normal rat to growth hormone is conditioned by the physiology of the individual bone and that growth hormone does not accelerate epiphyseal closure, any more than it prevents closure at the normal time.

The skeletons of giants produced by chronic administration of growth hormone are characterized by many disproportions due to this fact that some of their epiphyses close without the bones' exceeding normal dimensions, whereas others remain open and the bones continue to grow beyond normal dimensions. The possibility that these disproportions may be interpreted as similar to acromegalic changes in man needs further analysis and will be discussed in relation to the response of hypophysectomized rats to growth hormone.†

Growth hormone in hypophysectomized rats. On injection of growth hormone into hypophysectomized rats, increase both in weight and skeletal dimensions occurs promptly. Hypophysectomized rats are more sensitive to growth hormone than are normal rats; smaller doses are required to produce a given increment than are required in the normal. All epiphyseal cartilages present respond, becoming more cellular and wider. The increases in epiphyseal cartilage width, within certain limits of dose and time, are proportional to the dose. The reliability of this response has furnished the basis of a very satisfactory method of assay of the hormone.[25, 21, 26] The test is specific in the sense that the growth hormone is the only hormone which widens the plate in proportion to dosage.‡

The resumption of growth of epiphyseal cartilages is accompanied by removal of the sealing lamina of bone, the reëstablishment of mechanisms of erosion, the removal of old, heavy trabeculae, and deposition of new bone in delicate trabeculae. Reëstablishment of the youthful picture thus occurs, replacing the aged appearance characteristic of the bone of hypophysectomized rats.[11]

It will be recalled that in normal rats growth hormone neither delayed nor accelerated epiphyseal closure. In hypophysectomized rats epiphyseal

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* To appear in Anat. Rec., 1950, *107*, no. 4.

† The most noteworthy change simulating acromegaly was the excessive growth of the mandible which resulted in dental malocclusion and marked distortion of condylar form.

‡ Small increases in cartilage width, below the unit defined for growth hormone, can be produced by other hormones. None produces continued growth, or growth proportional to dose. (Marx *et al.*[42]).

closure is already postponed indefinitely by the operation and therefore no question arises of a further delay with growth hormone. Whether growth hormone hastens epiphyseal closure is very doubtful. As increased dimensions result from injection of growth hormone, this is a part of the general problem as to whether epiphyseal closure occurs when bones reach their definitive length, or when a characteristic chronological age is reached, or after a definite period of activity has elapsed. Silberberg and Silberberg[57] have taken the position that epiphyses close when, as a result of endogenous or exogenous stimuli, the bones have reached full size.

In order that experiments in which growth hormone is administered may contribute to the solution of this problem, it is extremely important that growth hormone preparations should be free of extraneous biologically active substances. Traces of thyrotropic hormone in such preparations would undoubtedly have a differentiating effect on the bones. In the studies reported here, growth hormone has been used which was pure by physico-chemical standards, and known to be free of thyrotropic effects at many multiples of the minimally effective dose.

It would appear that growth hormone does not hasten closure, but that the time of closure, as well as the responsiveness of bones to growth hormone, is dependent on, or related to, the normal physiology of the bone as reflected in normal time of closure. Although all epiphyses present at hypophysectomy were reactivated by growth hormone, the continuance of the response of each bone followed the same pattern as the epiphyseal closure. Epiphyseal discs which persisted until late in life—such as that at the proximal end of the tibia—continued to respond as long as the hormone was administered, and the bones reached a size in excess of normal, whereas epiphyses which normally close early responded only until the normal size of bone was attained.

The latter situation may be illustrated by the reaction of the metacarpal of the hypophysectomized rat during the chronic injection of growth hormone (Becks *et al.*,[16] p. 182, footnote). Epiphyseal closure in the metacarpal was delayed, as in the uninjected control, far beyond the normal age of closure (110 days) though the sealing lamina of bone was absent and the epiphysis responded to the hormone until normal length was attained. Nor did the epiphysis close promptly once the definitive bone length was achieved, 180 days after the normal time of closure, when rats were 285 days old. Instead, this epiphyseal disc remained for some time without further contributing to the length. (The epiphysis did eventually close, by 450 days, which it did not do in the uninjected controls.)

Hypophysectomized rats becoming gigantic as a result of injection of growth hormone (i.e., exceeding normal weight and length) developed many disproportions in the skeleton. These were even more marked than in the intact injected rats.[27] Part of these disproportions are certainly

attributable to the differences in responsiveness of the various epiphyseal centers. The relatively small paws of the growth hormone induced giant rats are one of the outstanding disproportions noted which can be attributed to this differential responsiveness of various epiphyses.*

Aside from the disproportion in the paws, other outstanding disproportions were present in the skull and pelvis. In the skull the disproportions were chiefly in the parts dependent on membranous bone growth. In the pelvis the sustained growth was not attributable to an epiphysis. In the scapula also, disproportions must have been due to bony additions from periosteal growth. The tuberosity of the humerus, which might be considered a periosteal excresence, surpassed in size anything seen in normal giants.

Growth hormone in thyroidectomized rats. That growth can be elicited in thyroidectomized rats by injection of growth hormone has been reported many times.[34, 108] Salmon[50, 51] questioned whether rats thyroidectomized on the first day of life could respond. Scow and Marx,[54] Scow *et al.*,[55] and Ray *et al.*[48] have found, however, that rats thyroidectomized at this early age responded by an increase both in body weight and length; bones throughout the body were stimulated. It is true these animals were relatively insensitive to the hormone, 25 times as much being required as is necessary to induce similar growth in hypophysectomized rats. However, no maturation of the skeleton resulted from the injection of growth hormone into thyroidectomized rats. This could be shown to optimal advantage in those rats thyroidectomized on the first day of life, as both growth and osseous maturation were so markedly retarded by the operation.

Growth hormone in thyroidectomized-hypophysectomized rats. Rats in which both thyroid and pituitary were removed responded to growth hormone like thyroidectomized rats. They increased in weight and length, but were relatively insensitive to the hormone. Furthermore, no maturation of bone occurred; bone age remained at 18 days as in the doubly operated controls.

* Bones with one epiphysis, and therefore dependent for growth only on one locus which normally fuses early, grew to normal dimensions and then ceased to grow. The paws, which contain many bones of this type (metacarpals, phalanges, and at least some of the tarsals, e.g., the calcaneous) behaved in this way, with the result that the paws were never enlarged in hypophysectomized giants. The other long bones have two foci of growth. Usually one focus (such as that at the distal tibia and proximal radius) closes early. Such epiphyseal discs ceased to respond to growth hormone during chronic injection. The second epiphysis of such long bones, characterized by delayed closure, remained responsive to growth hormone and therefore growth continued to excess in such bones.

It is puzzling to reconcile the occurrence of large feet and hands in human giants with the unenlarged paws of chronically injected rats (normal or hypophysectomized). It cannot be said that the difference is due to lack of time for influencing the bones of the paws because of early closure of epiphyses. The metacarpals attain the same length whether chronic injections are started at 21 days or later. In this sense the rat is a more highly specialized animal than man.

Growth hormone is effective in the absence of the target organs. The response to growth hormone of rats deprived of other endocrine organs has also been tested. Hypophysectomized-adrenalectomized rats were found to respond to growth hormone with the same sensitivity as hypophysectomized rats.[39] Hypophysectomized-adrenalectomized-thyroidectomized rats also responded well to growth hormone (Simpson, Li, and Evans unpublished). Hypophysectomized-thymectomized rats were injected with growth hormone and were found to be as sensitive in their response as hypophysectomized rats.[40] This was of interest as it was known that the thymus enlarged in either normal or hypophysectomized rats injected with this hormone. Gonadectomized rats also respond well to growth hormone.[22] It may then be safely said that growth hormone does not act through any of the known target organs.

TABLE 6

SKELETAL AGE OF FEMALE RATS HYPOPHYSECTOMIZED AT 21 DAYS OF AGE AND INJECTED WITH THYROXIN BETWEEN 30 TO 60 DAYS OF AGE

Treatment	*Skeletal age*		*Advance in skeletal age*
	At 30 days	*At 60 days*	
Hypophysectomized untreated	29	42	13
Hypophysectomized + Thyroxin 2.5 μg per day	29	55	26
Normal untreated	30	60	30

After Ray *et al.*[47]

Thyroxin therapy

Thyroxin in normal rats. Thyroxin injected into normal rats (at 5μg doses) caused no increase in body length, but increased even further the growth resulting from growth hormone.[24] Osseous differentiation has also been shown to be accelerated by thyroid hormone and premature aging of the skeleton results. Smith and McLean[61] demonstrated this for the tibia in the rat. Premature closure of the epiphysis of the intact rat's metacarpal has been observed in this laboratory; the disc disappeared at 82 days, the average time of disappearance being 110 days (H. V. Christensen and I. M. Carlson, unpublished). Silberberg and Silberberg[58] showed premature epiphyseal closure in adult mice. Noback *et al.*[44] were able to show that thyroxin advanced osteogenesis in newborn rats.

Thyroxin in hypophysectomized rats. Thyroxin injected into hypophysectomized rats caused barely detectable growth.* The outstanding result of thyroxin injection was increased differentiation of bone. Maturation occurred up to normal for the age, even though the animal remained dwarfed.[3, 47] The advance in skeletal age is shown in Table 6.

Thyroxin reduced the width of the epiphyseal plates so that they were even narrower than in hypophysectomized controls; erosion of cartilage dominated, leading to the increased bone age. The formation of a lamina of bone sealing the cartilage was prevented (Ray *et al.*,[47] also C. P. Williams, unpublished), and resorption of such laminae occurred if already formed—even though the sealing bone had been present for long periods.[11]

Thyroxin with growth hormone

When thyroxin was injected into hypophysectomized rats in combination with growth hormone, both growth and maturation occurred. A very delicate balance of dosages is necessary, otherwise one or the other effect predominates. Smith[62] first described this synergic action between thyroid hormone and growth hormone and it has been confirmed many times (e.g., Evans *et al.*[24]). Histological analyses of the skeletons showed that greater increases in dimensions of the bones resulted than after either treatment alone, and that the combination also caused a more marked increase in chondrogenesis and osteogenesis.[8] Thyrotropic hormone from the pituitary showed the same synergic action with growth hormone in hypophysectomized rats.[41]

Thyroxin, and thyroxin with growth hormone, in thyroidectomized rats. Thyroxin injected into thyroidectomized rats led to spectacular resumption of growth (increase in size)—a growth as marked as that caused by growth hormone in hypophysectomized rats. Differentiation of the osseous system also occurred, leading to normal rate of increment of skeletal age. In rats thyroidectomized on the day of birth a dose of thyroxin of only 2.5 to 3μg caused an advance in differentiation so that at 60 days of age, the bone age attained was 56 days even though therapy had not been instituted until 30 days of age.[17, 48, 55]

As thyroxin therapy is accompanied by repair of the abnormal pituitary of the thyroidectomized rat, it may be assumed that the increase in size was caused by resumption of secretion of pituitary growth hormone.

Thyroxin, and thyroxin with growth hormone, in thyroidectomized-hypophysectomized rats. In rats both thyroidectomized and hypophysect-

* Doses of thyroxin of 5 to 7 μg, doses which are marginally toxic, caused no growth. Doses of 2 to 3 μg caused a small amount of growth. As usual, in interpreting these minimal growth effects one must distinguish between barely detectable and transient increases and growth which is proportional to dose and which continues as long as the substance is administered.

omized, on days 1 and 20 respectively, thyroxin administered from day 30 caused slight but significant increases in body weight and length, also increase in the length of individual bones. Differentiation of the skeleton was resumed at a normal or greater than normal rate.* The body growth

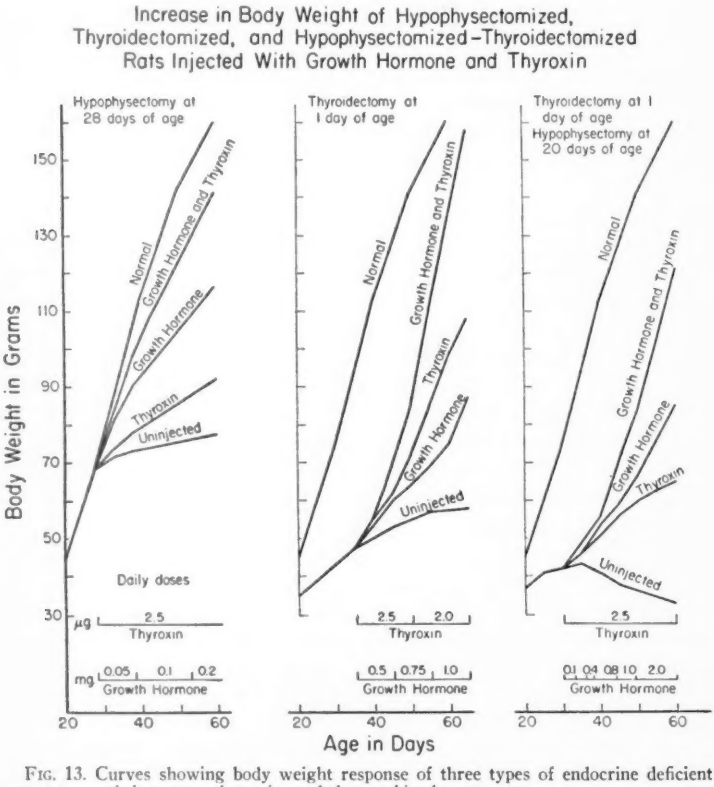
Increase in Body Weight of Hypophysectomized, Thyroidectomized, and Hypophysectomized–Thyroidectomized Rats Injected With Growth Hormone and Thyroxin
Hypophysectomy at 28 days of age
Thyroidectomy at 1 day of age
Thyroidectomy at 1 day of age
Hypophysectomy at 20 days of age
Body Weight in Grams
Normal
Growth Hormone and Thyroxin
Growth Hormone
Thyroxin
Uninjected
Daily doses
μg 2.5 Thyroxin
mg 0.05 0.1 0.2 Growth Hormone
2.5 2.0 Thyroxin
0.5 0.75 1.0 Growth Hormone
2.5 Thyroxin
0.1 0.4 0.8 1.0 2.0 Growth Hormone
Age in Days

FIG. 13. Curves showing body weight response of three types of endocrine deficient rats to growth hormone, thyroxin, and the combined treatment.

resulting from injection of growth hormone was greater than that from thyroxin, but growth hormone caused no skeletal differentiation. The combination of growth hormone with thyroxin was more efficient than either hormone alone, causing a normal or greater than normal rate of growth with maturation.[70] Both the hormones necessary for coordinated growth with maturation had to be supplied in this type of animal to elicit the

* Skeletal maturation may even occur at a greater than normal rate as indicated by the closure of the medial epicondyle of the humerus at 60 days though the normal age is 100 days.

complete response, since, unlike the thyroidectomized animal, the thyroidectomized-hypophysectomized rats could not react under the stimulus of thyroxin to produce endogenous growth hormone.

The graphs of Figs. 13 and 14 serve to illustrate the relative effectiveness of thyroxin, growth hormone, and the combination of thyroxin and growth

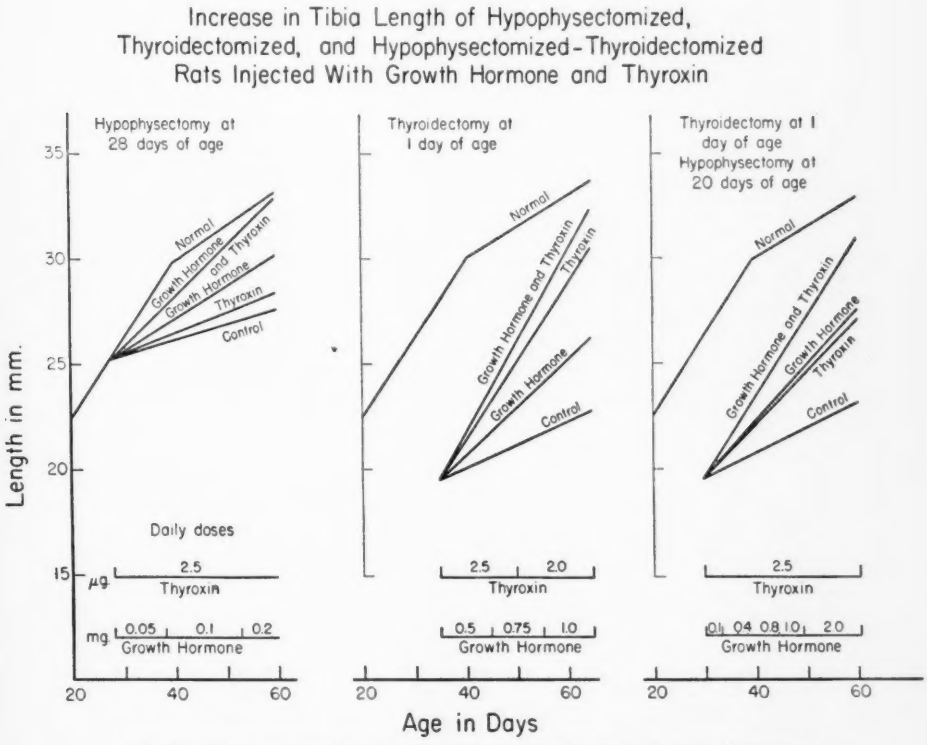

FIG. 14. Curves showing increase in length of the tibia of three types of endocrine deficient rats to growth hormone, thyroxin and the combined treatment.

hormone in thyroidectomized, hypophysectomized, and doubly operated rats. The first chart (Fig. 13) shows the increase in body weight, the second the increase in bone length (tibia, Fig. 14).

Figures 15-20 show the differential histological response of the humerus of the thyroidectomized-hypophysectomized rat to injection of thyroxin, growth hormone, and the combination.

Emphasis has been placed in this paper on the roles of growth hormone and thyroxin on growth of the skeleton—i.e., their effects on increase in

size and maturation—and no complete discussion has been attempted of the action of other pituitary hormones or of their target organ hormones. Nevertheless, a few other outstanding hormonal effects on growth must be mentioned, as the basic action of growth hormone and thyroxin is markedly modified by other hormones. Even though many of these interrelations are still obscure, it would be a mistake not to touch on some of the better established ones.

Effect of adrenocorticotropic hormone and cortical steroids. In normal rats adrenocorticotropic hormone (ACTH) caused retarded body growth.[7, 43] Retardation of both chondrogenesis and osteogenesis occurred, the epiphyseal plates were narrower than is normal, though not as narrow as in hypophysectomized rats. This antagonism to growth was shown to be effective through the cortex of the adrenal as it was not given in the absence of the cortex.[28] Cortical steroids have been shown to have a similar antagonistic action on growth.[34]

In hypophysectomized rats ACTH did not further decrease chondrogenesis or osteogenesis when given alone, but when injected simultaneously with growth hormone it was antagonistic to the growth promoting effects.[8] The body weight increase and epiphyseal disc widening anticipated on injection of growth hormone did not occur (Becks *et al.*, 1944).† No attention was devoted to the effect of ACTH or cortical steroids on maturation of the skeleton.*

† Since writing this, roentgenographic studies in this laboratory of the skeletons of intact and of hypophysectomized rats treated from 22 to 60 days of age with doses of ACTH which produced growth stasis have shown no appreciable effect of this hormone on skeletal maturation.

* This antagonism of ACTH to growth hormone may be associated with opposite and opposing action on nitrogen metabolism, ACTH wasting nitrogen and growth hormone conserving it.

PLATE IV

Response of the humerus of thyroidectomized-hypophysectomized rats to thyroxin, growth hormone, and the combination. Specimens taken at 60 days of age except as noted. (H & E stain, x9.)

FIG. 15. Normal rat 18 days of age, "bone age" control. The main epiphyseal ossification center is present.

FIG. 16. Thyroidectomized day 1, subsequently hypophysectomized day 20. Only the main epiphyseal ossification center is present.

FIG. 17. Both operations as described above, treated with thyroxin, 2.5 μg daily, day 30-60. The main epiphyseal cartilage plate and the plate in the medial epicondyle are both perforated.

FIG. 18. Both operations. Growth hormone, dose increasing from 0.1 to 2.0 mg daily, day 30-60. Increase in size of the bone, but no further advance in ossification beyond the doubly operated control.

FIG. 19. Both operations. Growth hormone and thyroxin (both as above). The main epiphyseal cartilage plate has disappeared. That in the medial epicondyle is intact and active.

FIG. 20. Normal rat 60 days of age, chronological age control. The main epiphyseal cartilage plate has disappeared. The plate in the medial epicondyle is still active.

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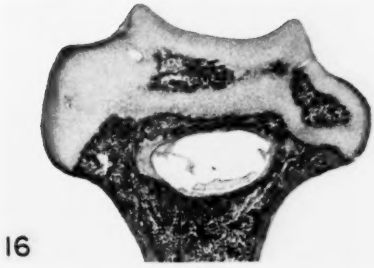

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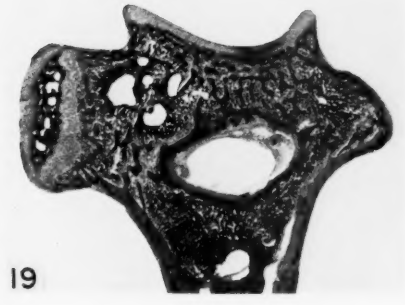

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PLATE IV

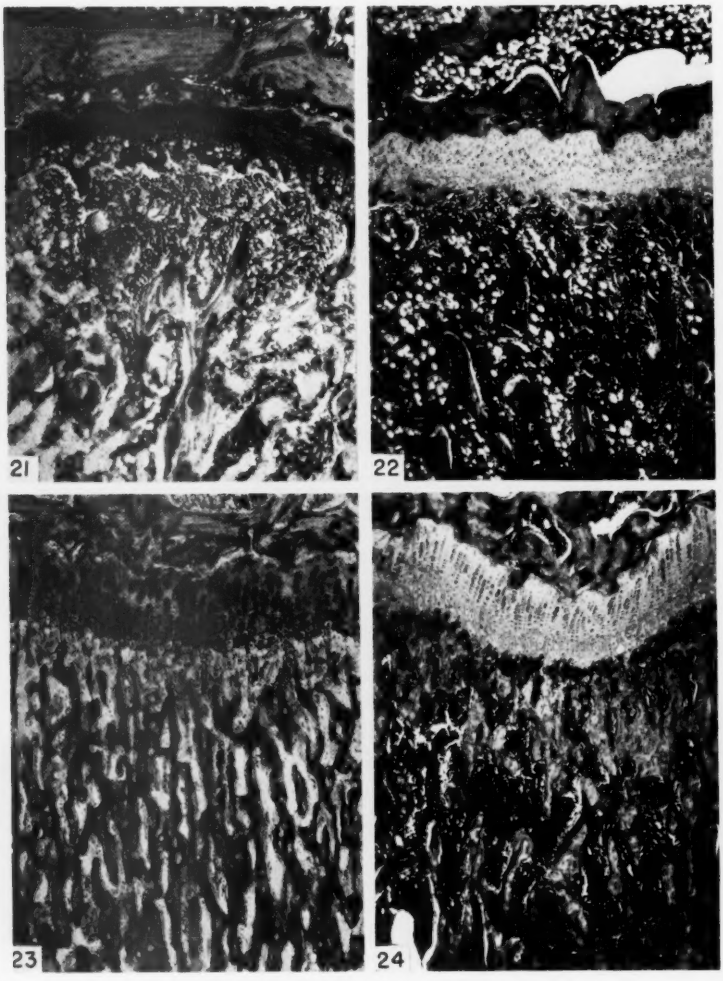

PLATE V

Effect of testosterone. Testosterone has been shown to stimulate growth in normal animals of several species, including the human being. In the absence of the pituitary the rat gave no significant increase in weight or length when injected with testosterone (Simpson *et al.*[60]; also G. G. Cayler, unpublished, and C. P. Williams, unpublished). No significant stimulation of the growth of bones has been noted, with the exception of the os penis which has been shown by Thyberg and Lyons[68] to respond to testosterone rather than growth hormone. Testosterone caused differentiation as well as growth of this sexually dimorphic bone.†

Some augmentation of the effects of growth hormone by testosterone has been reported to occur in hypophysectomized rats.[60]

Testosterone combined with thyroxin therapy in the hypophysectomized rat did not give any effects other than those anticipated from thyroxin alone (C. P. Williams, unpublished).

From these results it might be inferred that the growth-promoting effects of testosterone observed in normal animals, but not in the absence of the pituitary, are due to stimulation of the pituitary, or perhaps to synergism with pituitary growth hormone. The expected synergism with thyroxin was not observed, and the synergism with growth hormone under the conditions so far tested was slight. The conservation of nitrogen by testosterone occurs in the absence of the pituitary, so that nitrogen conservation cannot be the only factor in the growth which it initiates.[30]

Effect of estrogen. The smaller size of the normal female rat than the castrate female may be interpreted as due to the antagonistic action of endogenous estrogen on growth. So also the smaller size of growth-hor-

† Turner *et al.*[66] showed that castration of day-old male rats was without effect on their skeletal age (except the os priapi and ischial epiphysis). They showed roentgenographically that testosterone did not increase skeletal age of normal or castrate males but that it did accelerate development of the os priapi. G. G. Cayler in this laboratory (unpublished) has found that the pelvis of the rat similarly increases in size and differentiates with testosterone.

PLATE V

Comparison of the effects of lactogenic hormone with those of estrogen. Proximal epiphyseal cartilage plate of the tibia (H. & E. stain, x38.)

FIG. 21. Hypophysectomized 54-day-old female rat treated with estradiol dipropionate for 18 days (10 mg pellet implanted subcutaneously at weekly intervals). No difference from the control is to be noted.

FIG. 22. Untreated hypophysectomized 40-day-old female rat, 21 days postoperative.

FIG. 23. Normal female rat treated with estradiol dipropionate for 18 days. (10 mg pellet implanted subcutaneously at weekly intervals). Trabeculae are numerous and sturdy.

FIG. 24. Hypophysectomized 40-day-old female rat injected with lactogenic hormone for 10 days (day 11-21 postoperative). Note resemblance to the normal estrogen treated.

mone-injected normal females compared with injected castrate females may be interpreted as due to antagonism of estrogen to growth hormone.[53]

The effects of injected estrogen on bone are very much more marked in some species of animals than in others. In mice (and pigeons) estrogen causes complete filling of the medullary cavity with bone (Gardner and Pfeiffer[59]). In rats, injected estrogen has less effect on bone structure than in mice. There is, however, an increase in amount of trabecular bone and a decrease in cartilage width.[58] Urist, Budy, and McLean[68] consider that the mechanism in the rat is inhibition of resorption of bone, while in the mouse estrogen both stimulates endosteal ossification and inhibits resorption. In hypophysectomized rats no response of bone has been observed following treatment with doses of estrogen known to be effective in the normal.[37] The estrogen was administered immediately after operation and the resorption of trabecular bone characteristic of the immediate post-hypophysectomy period was not prevented. From this one can infer that in the rat, at least, the response to estrogen is mediated through the pituitary.

The effect of estrogen on skeletal ageing in the absence of the pituitary has not been adequately studied.

Effect of lactogenic hormone. Lactogenic hormone does have a marked effect on bone structure in hypophysectomized rats. The bony trabeculae found in the medullary cavity not only are not resorbed as usual after operation but are actually heavier than normal (Figs. 21-24) (Simpson, Asling, Becks, Evans, unpublished). The changes were quite comparable to those observed in normal rats injected with estrogen, suggesting that the mechanism for estrogen action in normal rats is through the pituitary, the estrogen stimulating the pituitary to produce more lactogenic hormone.*

While assuming that the growth and differentiation of the skeleton is primarily under the control of growth hormone and thyroxin, due care has been taken to stress the importance of many other factors which influence growth. Diet and other environmental factors have been mentioned, and lastly, the other endocrine influences. Though the parathyroids have been neglected, there has been no intent to minimize the importance of the hormone from this organ on mineral metabolism and hence on bone formation and resorption. The role of ACTH and cortical steroids, and of testosterone, in influencing nitrogen metabolism and hence the formation

* This interpretation is not without supporting evidence. A higher content of lactogenic hormone was shown in the pituitaries of estrogen-treated rats when tested by implanting the pituitary tissue in the crop wall of pigeons. Furthermore, the large persistent functioning corpora lutea of estrogen-treated normal rats may be interpreted as due to increased output of lactogenic hormone—through its luteotrophic action (Simpson and Evans, unpublished). Work with parabionts has given further convincing evidence of increased pituitary production of lactogenic hormone under the influence of estrogen.[85]

of bone protein matrix is equally inescapable. No adequate investigation has been made of these accessory endocrine factors to understand which of these influence only bone size, and which influence maturation, i.e., which of them act with or against growth hormone on the one hand or thyroxin on the other. Nor do we understand how they relate to proportionate or disproportionate growth of the various parts of the osseous system or to the deceleration or cessation of growth.

Summary

This account has largely been confined to analysis of the growth and differentiation of the osseous system in the rat under the influence of pituitary hormones and pituitary target organ hormones, stress being laid on studies to which our group has contributed. No attempt has been made to give a general review of osteogenesis.

As all the pituitary hormones, directly, or indirectly through their target organs, influence bone growth, it is important to stress the necessity of using pure hormones in any such study designed to disentangle the effects of individual hormones. It should be stressed particularly that the growth hormone preparations whose effects are here reported were pure, judged by physico-chemical criteria, and were free of biologically active contaminants at many multiples of the levels necessary for stimulation of growth.

The clearest evidence of dissociation of the phenomena of increase in size from differentiation in the skeletal system has been obtained from the study of osteogenesis in the animals deprived of the pituitary or thyroid, and from replacement studies with pituitary growth hormone and thyroid hormone.

After hypophysectomy in the young rat, growth and differentiation of the skeleton soon ceased. The skeletal age attained varied with the age at operation, being progressively less as the operation was performed in younger and younger animals. The period during which maturation continued after operation was, however, constant, being about 3 weeks, regardless of whether the rats were hypophysectomized at 28 days or 6 days of age. The capacity to increase in size was lost at a constant age, approximately 30 days chronological age.

No endocrine deficiency was produced in which growth hormone was not effective in promoting increase in size (hypophysectomy, thyroidectomy, adrenalectomy, the three operations concurrently; hypophysectomy-thymectomy or gonadectomy), i.e., growth hormone does not act through known endocrine organs.

The effect of growth hormone on the skeleton was chiefly to increase its size. Bones increased in length and diameter. All epiphyseal centers of ossification responded to growth hormone unless at the onset of therapy

they were too near their normal time of closure. Growth hormone in normal rats neither hastened epiphyseal closure nor delayed it. In hypophysectomized rats where epiphyseal closure was already postponed, growth hormone did not cause further delay. In fact, closure took place during its administration, though tardily.

The gigantism induced by growth hormone in normal, and especially in hypophysectomized rats, was characterized by many disproportions which appeared to be related to differences in responsiveness among epiphyses, which in turn was related to normal closure time of these epiphyses. Bones with early closing epiphyses closed without undergoing excessive growth, whereas bones with late closing epiphyses continued to grow as long as growth hormone was administered. The distortions characterizing growth hormone induced gigantism were also due to continuation of membranous or periosteal bone formation.

Thyroxin accelerated maturation of skeleton in normal rats; closure of epiphyseal plates occurred before adult size was reached. In the hypophysectomized rat also the effect was chiefly to promote differentiation or maturation. The most complete replacement in this deficient animal was therefore obtained by combinations of the two hormones, growth hormone and thyroxin.

Thyroidectomized rats reacted to growth hormone by increase in skeletal size, but showed no acceleration of maturation. Thyroid hormone in the thyroidectomized rat was, however, almost as effective in causing growth with maturation as the combination of growth hormone and thyroxin was in hypophysectomized animals.

No other hormones tested, alone or in combination, have stimulated significant amounts of growth, although other hormones are known to have important effects on skeletal growth—adrenocorticotropic hormone (or cortical steroids) and estrogens inhibiting growth and being antagonistic to growth hormone, and testosterone accelerating growth in the presence of the pituitary.

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THE EFFECT OF ANTIHISTAMINICS ON MICE HYPERSENSITIVE TO H. PERTUSSIS VACCINE*

I. A. PARFENTJEV

In connection with several recent publications[1,2] on reaction in children after injection of H. pertussis vaccine, it might be of interest to report our own observations on the action of antihistaminic drugs on mice sensitized by this vaccine.

In a previous publication[8] it was reported that treatment of mice with H. pertussis vaccine renders these animals highly sensitive to histamine. These findings were extended in a recent paper by Parfentjev and Schleyer.[10] Dependent on the vaccine dose and the mice selected for this type of experiment, the LD50 of histamine in sensitized mice was found to be 1/30[8] to 1/120[10] that of non-sensitized animals. It has also been shown[8] that antihistaminic drugs, e.g. Bromothen [N,N-dimethyl-N'(2-pyridyl)-N'(5-bromo-2-thenyl)-ethylene-diamine hydrochloride],[5] counteracted the histamine effect in sensitized mice, thus indicating that this technique might be used for the evaluation of anti-histaminic agents. A further advantage of using experimentally sensitized mice might be seen in the fact that the immunizing effect of H. pertussis vaccine also produced a hypersensitiveness of the animals to H. pertussis protein-antigen.[9] This offered the possibility of measuring the effect of antihistaminic drugs in mice against both histamine shock and anaphylaxis using the same sensitization in both instances.

In order to determine the usefulness of the mouse assay in the evaluation of antihistaminics, a number of experiments were carried out with the antihistaminic drug Thephorin, described by Lehmann[3] and Lehmann, Randall, and Hagan;[4] this substance, the tartrate of 2-methyl-9-phenyl-2,3,4,9-tetraphydro-1-pyridindene, appeared interesting because it bears no chemical relationship to any existing histamine antagonist.

The results of our experiments are presented in this paper.

Materials and methods

All experiments were carried out in white mice of 22-26 gm. originating from one mouse colony.

Sensitization. The same H. pertussis vaccine and the same dosage were used as described before.[10] The sensitizing dose was given intra-abdominally.

Challenging injections. In the tests mice were used at the peak of sensitivity. It was known from our preliminary experiments that the highest sensitivity toward histamine was obtained 4-5 days after the administration of vaccine, while the optimal anaphylactive hypersensitiveness to H. pertussis protein was observed after 11-12 days.

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Histamine was given intra-abdominally as a solution of the diphosphate. For the challenging injection of the antigen we applied a sterile detoxified formalin filtrate of a culture of H. pertussis (detoxified H. pertussis antigen). This antigen, which contains the proteins and other constituents of the organisms, was not toxic for normal mice but produced anaphylaxis and killed sensitized animals with the symptoms of acute shock. One-half ml. of this antigen was injected intravenously into the sensitized mice.

Protection by Thephorin. Thephorin was injected intra-abdominally 15 minutes before the challenge with histamine. If Thephorin was given *per os,* two treatments of the drug were administered by stomach tube with a one-hour interval between them. Four and a half hours after the second medication the challenging dose of histamine or antigen was injected.

Each test was performed on 2-4 mice simultaneously and was repeated on animals from several experimental lots of sensitized mice. Altogether 200 mice were used in this study.

Experimental

The results of the experiments are given in Tables 1 and 2.

Table 1 shows that the standard dose of 25 mg. Thephorin/kg. protected the majority of sensitized mice against 12 lethal doses of histamine, given

TABLE 1

PROTECTIVE EFFECT OF THEPHORIN TOWARDS HISTAMINE SHOCK IN MICE SENSITIZED BY H. PERTUSSIS VACCINE

Thephorin dose: 25 mg./kg. intra-abdominally.
Interval between Thephorin dose and histamine injection: 15 minutes.

Histamine diphosphate mg./kg.	*Number of lethal doses**	*No. surviving mice / No. injected mice*
125	5	6/7
300	12	7/10
500	20	1/6

* Based on 25 mg. histamine diphosphate/kg. as *one* lethal dose.

15 minutes later. There was no protection aganist 20 lethal doses. Oral administration of Thephorin (Table 2) also exerted a striking effect on the histamine shock as well as on the anaphylactic response to the specific H. pertussis antigen, if higher doses (50-100 mg./kg.) of this antihistaminic were given. Higher doses were necessary because of the long interval of 4½ hours between the administration of Thephorin and the injection of the challenging dose of histamine used in mice as compared to guinea pigs.

The protective effect of oral Thephorin toward histamine was best shown by a comparison of the LD50 of histamine diphosphate in sensitized mice with and without Thephorin. By using the cumulative method of Reed and Muench[11] for the calculation of the LD50 the following values were obtained:

LD50 of histamine diphosphate injected intra-abdominally in sensitized mice:

Without pre-treatment	:	< 25.0 mg.
4½ hours after 2 x 100 mg. Thephorin	:	155.7 mg.
4½ hours after 2 x 50 mg. Thephorin	:	89.1 mg.
4½ hours after 2 x 25 mg. Thephorin	:	< 50.0 mg.

These figures indicate that Thephorin, given *per os* in doses of 2 x 50 mg./kg. and 2 x 100 mg./kg. protected for a period of more than 4 hours against doses of histamine at least 3.5 and 6.2 times greater than the 50% lethal dose.

The higher dose of Thephorin (2 x 100 mg./kg.) also exerted a marked influence on the anaphylactic shock produced by intravenous injection of the antigen into the sensitized mice. The results of this experiment can only

TABLE 2

PROTECTION OF SENSITIZED (H. PERTUSSIS VACCINE) MICE TOWARD HISTAMINE SHOCK AND ANAPHYLACTIC SHOCK (H. PERTUSSIS ANTIGEN) BY ORAL ADMINISTRATION OF THEPHORIN

Challenging agent	*Dose mg./kg.*	*Untreated controls*	*No. surviving mice/No. injected mice Thephorin*		
			2 x 100 mg./kg.	*2 x 50 mg./kg.*	*2 x 25 mg./kg.*
Histamine diphosphate[a]	25	7/29	2/2		
	50	1/12	8/11	6/8	1/4
	125	....	9/14	5/15	1/4
	300	....	3/8		
H. pertussis antigen[b]	0.5 ml.[c]	1/11	7/13		

[a] Injected intra-abdominally.
[b] Injected intravenously.
[c] 0.5 ml. per mouse.

be considered as of borderline significance, while the corresponding observations with histamine were found to be not due to chance ($p = 0.01$ or less).

Discussion

The experiments described above bring forth evidence that the antihistaminic drug Thephorin is effective in protecting sensitized mice against histamine shock produced by 6-12 multiples of the minimal lethal dose of histamine. Since similar effects were previously described for Bromothen, it seems likely that other antihistaminic agents will also be effective under these experimental conditions. The sensitization of the mice by the pretreatment with H. pertussis vaccine seems essential, since according to Mayer and Brousseau[8] there was no protective effect of Pyribenzamine and

Benadryl against the high doses of histamine required to produce toxic effects in normal mice. In the sensitized mice, on the other hand, considerably lower doses of histamine exerted a toxic effect and could be counteracted by the antihistaminic drug. The histamine doses required by the sensitized animals were, however, still considerably higher than those in guinea pigs (the minimal lethal dose of histamine diphosphate of 25 mg./kg. sensitized mouse represents about 30 times the LD50 for guinea pigs) and correspondingly higher doses of Thephorin had to be given in mice.

Despite certain quantitative differences of drug dosage, the protective effect of Thephorin in sensitized mice appeared similar to that observed in guinea pigs. Moreover, mice sensitized by vaccine seem to be particularly suitable for studies of bacterial allergy since they can be tested for histamine shock and anaphylaxis.

Concluding this discussion we wish to mention recently published observations of Globus and Kohn[2] and Byers and Moll[1] on the reaction in children during immunization against whooping cough. In experiments with the immunization of rabbits with egg albumen Meier and Buchner[7] made very interesting observations on the application of an antihistaminic drug. The authors protected sensitized rabbits with an antihistaminic drug —Antistin—and so were able to reinject such animals with larger booster doses of antigen—egg albumen—to obtain serum with a higher antibody titer.

Summary

In experiments with Thephorin it was possible to demonstrate the inhibiting effect of this drug on both histamine shock and anaphylaxis in mice hypersensitive to H. pertussis vaccine.

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THE ROLE OF EPINEPHRINE IN THE SECRETION OF THE ADRENAL CORTEX*

H. GERSHBERG,† E. G. FRY, J. R. BROBECK, AND C. N. H. LONG

In 1943 in a review of the factors producing adrenal cortical hypertrophy, Tepperman, Engel, and Long[31] suggested that, "The cortical hormone may inhibit the adrenotropic activity of the anterior lobe, and the adrenotropic secretion may therefore increase if the cortical hormone concentration in the body fluids falls. A fall in the cortical hormone concentration may be brought about by an increase in the rate of inactivation ('utilization') of the hormone."

This suggestion was prompted by the observation of Ingle, Higgins, and Kendall[12] that the administration of cortical hormone to normal rats was followed by atrophy of their adrenal cortices, and that simultaneous administration of adrenotrophic hormone (ACTH) prevented such atrophy. The rapid inhibition of ACTH release by the administration of cortical hormone to normal animals has been adequately demonstrated by Sayers and Sayers.[24] These authors have shown that the depletion of the adrenal ascorbic acid which normally follows exposure of animals to a variety of conditions and agents, and which is initiated by ACTH discharge, does not occur if adrenal cortical steroids are injected prior to the imposition of the previously effective agent.

In consequence they put forward a view almost identical with that expressed above, i.e., that a wide variety of circumstances and agents increase the rate of utilization of cortical hormone by the cells of the tissues. This soon leads to a reduction in the blood level of cortical hormone which acts as an effective stimulus to the release of ACTH, thus assuring a continued high level of adrenal cortical secretion. Conversely, any situation in which the blood level of cortical hormone rises to above normal levels brings about an inhibition of ACTH secretion, thus lessening the secretory rate of the adrenal cortex. In this way continued high and possibly deleterious blood levels of cortical hormone are prevented.

In 1945, Long and Fry[15] and, later, Long[13, 14] pointed out that many of the circumstances associated with the release of ACTH are those which

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† Senior Research Fellow of the National Institute of Health. Present address, Department of Medicine, New York University College of Medicine.

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for many years have been known to be also associated with an increased degree of activity of the autonomic nervous system and a concomitant release of epinephrine. They reported that the injection or infusion of epinephrine is also followed by a decline in adrenal ascorbic acid and cholesterol which fails to occur in hypophysectomized animals. It was suggested, therefore, that epinephrine is an important factor in the activation of ACTH secretion and may well serve as the trigger mechanism by which adequate amounts of adrenal cortical hormone are rapidly made available for the bodily needs of the moment.

While these studies were in progress, Vogt,[35, 38] using an ingenious technique by which the content of cortical hormone in the venous effluent blood of the gland of the dog could be determined, reported that epinephrine in physiological amounts is a potent stimulus of this secretion. The characteristics of this effect of epinephrine on the cortical secretion were, first, the rapidity with which the increased output of cortical hormone occurred, and secondly, the comparatively long period after cessation of epinephrine infusion that the high output of cortical hormone was maintained. The one significant difference from the results of Long, *et al.*, was the conclusion that this effect of epinephrine was independent of the presence of the pituitary—a conclusion based on one reported experiment on a decapitated dog.[38]

Sayers and Sayers,[35] although they also found that epinephrine injection is followed by adrenal cortical activation, are inclined to the belief that the effect of epinephrine is due to its capacity to increase the utilization of cortical hormone, and is thus only an indirect stimulus to ACTH secretion.

The present paper and that immediately following are concerned with reconciling the two important differences in the results of these three groups of investigators. The questions at issue are these: (a) Does epinephrine produce an augmentation of adrenal cortical secretion by its *direct* effect on the cells of the adrenal cortex, or is this effect mediated by a preliminary stimulation of ACTH secretion from the anterior lobe of the pituitary? (b) Since, in our experience, epinephrine only augments cortical secretion in the presence of the anterior lobe, the second question is concerned with the mechanism by which epinephrine provokes the secretion of ACTH. This might be (i) indirectly through an enhancement of cortical hormone utilization in the tissues, as Sayers and Sayers suggest; or (ii) by a direct effect on the cells of the anterior lobe that are responsible for ACTH formation and release. Data will be presented which appear to answer these two questions, and an outline will be given of the main components of the mechanism through which the autonomic nervous system and the adrenal medulla may increase the rate of liberation of adrenocorticotrophic hormone.

Methods

In this and the following paper two methods have been employed to detect the increased secretory activity of the adrenal cortex of albino rats. The first of these is the reduction in adrenal cholesterol and ascorbic acid that occurs when the blood level of ACTH is increased by natural or artificial means. The specificity of this response of the adrenal cortex to ACTH was first reported from this laboratory by Sayers, *et al.*[26] Since that time the method has been extensively used to detect adrenal cortical activation and has been adapted for use as an assay method by Sayers, Sayers, and Woodbury.[25] Actually, it is a measure of increased blood levels of ACTH, but the physiological coupling of this hormone with adrenal cortical secretion makes it equally applicable for estimation of the latter.

The second method depends on the capacity of the 11-oxy adrenal cortical steroids to cause a decrease in the number of circulating eosinophiles. This was first described by Thorn and his colleagues,[9] and is analogous to the reduction in circulating lymphocytes found by Dougherty and White[6] and of the monocytes described by Thompkins.[28] The eosinopenic response is a sensitive method for the detection of increased blood levels of these cortical hormones. The close relationship which exists between the decline in adrenal ascorbic acid and that of the circulating eosinophiles is described in detail in the paper which follows this.

Chemical. Analyses for ascorbic acid content of the adrenal glands were made by the method of Roe and Kuether.[23] Cholesterol was determined by the procedure of Sperry.[29] Liver and muscle tissues were analyzed for glycogen by a modification of the well-known Pflüger method. Blood samples were collected from the tail before and after the experimental procedures for the estimation of glucose,[18] lactic acid,[2] and plasma amino nitrogen.[10]

Hematological. Direct counts of blood eosinophiles were made on freely flowing samples of tail blood by the method of Randolph,[21] modified to stain only eosinophilic cells. A diluting fluid containing 0.05% phloxine* in 50% propylene glycol* was prepared freshly every few days by a 1:1 aqueous dilution of a 0.1% stock solution in 100% propylene glycol which was stored in the refrigerator. Mixing time was standardized to obtain uniform cell distribution in the 1:20 dilution of the blood sample. In our experience, drops from the middle portion of the pipette gave close agreement on both sides of two 0.2 mm.-depth Levy counting chambers. Selection of uniform diluting pipettes is recommended for satisfactory results. Variations in resting levels of circulating eosinophiles were encountered in spite of the above precautions. However, our range for the rat has never been as wide as that reported by Recant, Hume, Forsham, and Thorn,[22] and repeated counts every few days revealed similar values for any one animal. Figures obtained in this laboratory by both the Randolph[21] and Forsham, *et al.*[9] techniques ranged between 100-200 cells per cubic mm. for the normal rat.

Anesthesia. Warmed 1% solutions of sodium pentobarbital (Nembutal) in physiological saline were injected intraperitoneally; intact rats received 5 mg./100 g., while hypophysectomized, adrenalectomized, and other operated animals were given 3-4 mg./100 g. Whenever postoperative respiratory difficulty was anticipated, Evipal was

* Eimer and Amend, New York.

used as an anesthetic at a dose level of 7.5-10 mg./100 g. For local anesthesia a 1:20 dilution of 20% Novocain was employed.

Drugs. Epinephrine hydrochloride (Adrin, 1:1000, Sharp & Dohme) was used,* diluted with saline as noted in tables. Reduced glutathione (Paragon) in a concentration of 20 mg. % was added to protect the epinephrine from oxidation during administration. Intravenous, intraperitoneal, and intramuscular (gastrocnemius) infusions were made through a 26-gauge needle connected to a perfusion pump adjusted to deliver 0.5 ml. of fluid per 100 g. of body weight per hour.

Intraocular injections of epinephrine were done under light Nembutal anesthesia. The sclerocorneal junction of the rat's eye was nicked with a pointed scalpel blade, some aqueous humor was allowed to escape, and then 0.05 ml. of a 1:250,000 dilution of Adrin (0.2 micrograms) were slowly introduced with a fine gauge needle.

Adrenal cortical hormone used was either Wilson's extract or Upjohn's Lipo-Adrenal and was administered as noted in the tables. The desoxycorticosterone acetate injected was either Cortate (Schering)† containing 5 mg. in 1 ml. sesame oil, or a suspension of crystalline D.C.A.† prepared by dissolving 20 mg. in 1 ml. of warm absolute alcohol and by diluting this with 9 ml. of physiological saline. All adrenal cortical steroids were injected subcutaneously, in divided dosages if the volume was large.

Lilly's insulin (Iletin), U-40, was diluted 1:40 with saline so that 0.1 U. (0.1 ml.) per 100 gm. was injected intraperitoneally. For the intravenous route 0.05 U./100 g. were introduced into the femoral vein, which had been exposed under local Novocain anesthesia.

Ergotamine tartrate (Gynergen, Sandoz) containing 0.5 mg./ml. was injected subcutaneously in doses of 0.5 mg./100 g.

Preparation of animals. Sprague-Dawley male rats ranging from 200-300 g. were used. They were housed in a constant temperature room at 78-80° F. The diet was Laboratory Chow, except in the case of the rats with diencephalic lesions and those with transection of the spinal cord, where a high-fat diet was fed.‡ In the experiments described in this paper, all rats were fasted for 24 hours except where indicated in the tables. The studies in the second paper were made on non-fasted animals, primarily because repeated fasting brought about untoward loss of weight in the spinal and diencephalic animals. In every case, eosinophile counts were made at the same hour on each experimental day, and food was withheld during the observation period.

Hypophysectomy, adrenalectomy, adrenal enucleation *in situ*, orchidectomy, and splenectomy were all performed by standard procedures.[8] Adrenalectomized rats were maintained on physiological saline as drinking fluid, while adrenal demedullated animals were given saline to drink for the first postoperative week. Four to five weeks were allowed for cortical regeneration[11] before the latter animals were tested, and none was used after ten weeks.[3]

Spinal animals were prepared by transecting the cord at the level of the third thoracic (or, in a few instances, at the first lumbar) vertebra under Evipal anesthesia. By keeping the rats in a warm room (80° F.) and by establishing a routine of nursing care which included manual expression of the urine from the bladder twice daily until

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* Crystalline L-epinephrine bitartrate (obtained from the Sterling-Winthrop Research Institute as a gift to Dr. Desmond Bonnycastle), when infused intramuscularly in normal rats at a rate of 2 micrograms per 100 g. per hour, reduced the adrenal ascorbic acid concentration by 45%. However, L-arterenol bitartrate (Winthrop-Stearns) injected subcutaneously in normal rats in doses of 5 or 20 micrograms/100 g. had no significant effect on the adrenal ascorbic acid. The relative hypoglycemic effects of the two drugs were of the order reported elsewhere.

† Gift of the Schering Corporation.

‡ Lard, 34%, casein, 25.5%; sucrose, 25.5%; liver powder, yeast, and salt mixture, 5% of each by weight.

automaticity was effected, about 50% of these animals could be brought to a good state of health and nutrition. None was used which had not regained or surpassed his preoperative weight.

"Diencephalic" animals were prepared by placing electrolytic lesions in the thalamus and hypothalamus with the aid of the Horsley-Clarke stereotaxic instrument. Out of a number of rats operated upon and studied, six were selected for inclusion in the present report; they were in good health, gave no evidence of obesity or disturbances of temperature regulation, and were similar to the spinal and adrenal demedullated rats in that they consistently failed to secrete endogenous epinephrine.

Pituitary transplants were made into the anterior chamber of one eye of hypophysectomized rats (Evipal anesthesia) on the second day following the hypophysectomy. Pregnant female rats near term were used as donors. After slitting the sclero-corneal junction of the hypophysectomized rat's eye, one-half of the anterior pituitary tissue of the donor rat was introduced into the anterior chamber with the aid of a trocar. Aseptic technique was used throughout the operation.* One month later when the grafts were well established, experimentation was begun.

Stimulation of the central end of the sciatic nerve and exposure to cold have been described in earlier reports.[13, 14] Hemorrhage experiments were conducted as described by Sayers, *et al.*[27]

Bioassay of pituitary grafts. After the grafted eyes were removed from the hypophysectomized animals, the transplants were dissected out. Two grafts were extracted separately for adrenocorticotrophic assay. Saline to which had been added two drops of N/10 NaOH was used for extraction. Adrenocorticotrophic activity was tested according to the method of Sayers, Sayers, and Woodbury.[26] Parallel blood eosinophile tests were made. The remainder of the dissected grafts were pooled and extracted with saline for test of any gonadotrophic activity present. Assays were run on groups of immature rats and mice according to the technique of Evans, Varney, and Koch.[7]

Results

Effect of exogenous epinephrine. In their preliminary report, Long and Fry[15] stated that the subcutaneous or intravenous injection of epinephrine into normal rats brought about a decline in adrenal ascorbic acid and cholesterol. Similar injections into hypophysectomized rats were without effect on the levels of these adrenal constituents (Table 1). Since the time of their report, many more experiments have been carried out in which not only was the epinephrine administered by other routes, but the minimum quantity required to produce a fall in adrenal ascorbic acid and cholesterol has been roughly established.

The effects of epinephrine administered by the intravenous route to rats anesthetized with Nembutal are seen in Table 2. It will be observed that the intravenous injection of 0.9% NaCl over a period of sixty minutes is in itself sufficient to cause some fall in adrenal ascorbic acid but not in adrenal cholesterol. Such changes in adrenal ascorbic acid are also found after mild procedures such as incision of the skin and the insertion of a needle into the femoral vein. They would appear to be due to the reflex

* Dr. H. S. N. Greene carried out the implantations for us, according to the technique which he has recently described in detail (Yale J. Biol., 1950, *22*, 611-620).

secretion of epinephrine from the animal's own adrenal medulla. Indeed, in these experiments the infusion of a total of two micrograms of epinephrine in a corresponding quantity of normal saline produced no greater

TABLE 1

EFFECT OF THE SUBCUTANEOUS INJECTION OF EPINEPHRINE ON THE ADRENAL CHOLESTEROL AND ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS

Injection mg./100 g.	*Duration of experiment hours*	*No. of rats*	*Adrenal*	
			Cholesterol g./100 g.	*Ascorbic acid mg./100 g.*
		Normal rats		
none	...	23	4.05 ± 0.14	423 ± 13
0.02 x 1	1	6	3.80 ± 0.33	226 ± 24†
0.02 x 1	2	6	2.88 ± 0.28	233 ± 24
0.02 x 1	4	8	3.76 ± 0.19	252 ± 16
0.02 x 4	4	11	2.26 ± 0.22	216 ± 9
		*Hypophysectomized rats**		
none	...	13	5.60 ± 0.43	418 ± 26
0.02 x 4	4	9	5.34 ± 0.37	411 ± 13

* Three days postoperative.

† Data reported in the following paper show that the response within one hour following any subcutaneous injection is mainly a response to endogenous epinephrine from the animal's own adrenals.

TABLE 2

EFFECT OF INTRAVENOUS INFUSION* OF EPINEPHRINE ON THE ADRENAL CHOLESTEROL AND ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS (NEMBUTAL ANESTHESIA)

No. of rats	*Infusion*		*Total µg./100 g.*	*Time hrs.*	*Adrenal*	
					Cholesterol g. %	*Ascorbic acid mg. %*
23	Normal	None	..	..	4.05 ± 0.14	423 ± 13
6	"	Saline*	..	1	....	256 ± 25
6	"	"	..	2	4.01 ± 0.41	... ..
6	"	Epinephrine (1/250,000)	2	1	....	221 ± 20
6	"	" (1/25,000)	40	2	2.31 ± 0.18	104 ± 17
13	Hypox.	None	..	..	5.60 ± 0.43	418 ± 26
3	"	Epinephrine (1/25,000)	40	2	5.35	342

* Rate: 0.5 ml. fluid per 100 g. body weight per hour.

fall in adrenal ascorbic acid than the saline alone. On the other hand, the infusion of a total of 40 micrograms of epinephrine over a two-hour period caused a marked fall not only in adrenal ascorbic acid but also in adrenal cholesterol, the latter being an effect not obtained with saline alone.

In hypophysectomized rats even the intravenous injection of forty micrograms of epinephrine was without any such effect on the adrenal ascorbic acid or cholesterol, although the release of additional endogenous epinephrine and other factors associated with the operative technique were presumably present.

In an endeavor to prevent the release of endogenous epinephrine, further groups of normal and hypophysectomized rats were infused either intraperitoneally or intramuscularly (under Nembutal anesthesia) with solutions of epinephrine in saline. Control groups were infused with saline alone. Intraperitoneal injections of epinephrine (Table 3) in a total amount of 0.5-2.0 micrograms in one hour brought about significant falls

TABLE 3

EFFECT OF INTRAPERITONEAL INFUSION* OF EPINEPHRINE ON THE ADRENAL CHOLESTEROL AND ASCORBIC ACID OF THE NORMAL RAT (NEMBUTAL ANESTHESIA)

No. of rats	*Infusion*	*Total μg./100 g.*	*Adrenal*	
			Cholesterol g. %	*Ascorbic acid mg. %*
7	Saline*	....	4.00 ± 0.10	419 ± 18
9	Epinephrine (1/250,000)	2.00	3.78 ± 0.21	228 ± 12
5	" (1/500,000)	1.00	....	283 ± 34
8	" (1/1,000,000)	0.50	....	277 ± 34
4	" (1/5,000,000)	0.10	....	414 ± 19
11	" (1/10,000,000)	0.05	3.40 ± 0.20	326 ± 26

* Rate: 0.5 ml. per 100 g. body weight per hour. Duration of all infusions 1 hour.

in adrenal ascorbic acid but did not influence the cholesterol content of the gland. Amounts of epinephrine less than this, or the infusion of saline alone, was not followed by any consistent change in the ascorbic acid level.

The effects of intramuscular infusion are recorded in Tables 4 and 5. In these experiments only the changes in adrenal ascorbic acid were followed. It will be observed that the intramuscular infusion of saline alone over a period of one hour did not cause any fall in the ascorbic acid level. On the other hand, the infusion over a period of one hour of between 0.25 and 0.50 micrograms of epinephrine was associated with a highly significant decline in ascorbic acid. Amounts less than this did not cause any significant change in the level.

The time of infusion is of importance. In Table 4 it will be seen that whereas the infusion of 0.50 micrograms of epinephrine in a period of one hour was followed by a significant decrease, the infusion of the same quantity over a period of 15 minutes was without effect.

The absence of any such effects in hypophysectomized rats is recorded in Table 5. In these experiments the left adrenal was removed first, since (as

will be shown later) this procedure has no effect on the ascorbic acid content of the second gland in hypophysectomized animals. The epinephrine solution was then infused into the femoral vein for one hour, and the second gland removed. In no instance was there any significant change in the

TABLE 4

EFFECT OF INTRAMUSCULAR INFUSION* OF EPINEPHRINE ON BLOOD GLUCOSE AND ADRENAL ASCORBIC ACID OF THE NORMAL RAT (NEMBUTAL ANESTHESIA)

No. of rats	*Procedure* *Infusion*		*Total µg./100 g.*	*Time min.*	*Blood glucose mg. %*	*Adrenal ascorbic acid mg. %*
50	None		....	..	76 ± 2	422 ± 26
8	Saline		....	60	73 ± 1	452 ± 18
4	Epinephrine	(1/250,000)	0.500	15	106 ± 12	407 ± 15
4	"	"	1.000	30	98 ± 3	292 ± 7
6	"	"	2.000	60	90 ± 5	294 ± 12
4	"	(1/1,000,000)	0.125	15	68 ± 6	378 ± 9
4	"	"	0.250	30	65 ± 8	308 ± 35
6	"	"	0.500	60	.....	228 ± 16
7	"	(1/1,750,000)	0.290	60	.....	293 ± 16
7	"	(1/2,500,000)	0.200	60	.....	391 ± 18
2	"	(1/5,000,000)	0.100	60	.....	383

* Rate: 0.5 ml. fluid per 100 g. body weight per hour.

TABLE 5

EFFECT OF INTRAMUSCULAR INFUSION* OF EPINEPHRINE ON THE ADRENAL ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS (NEMBUTAL ANESTHESIA)

No. of rats		*Infusion*	*Total µg./100 g.*	*Adrenal ascorbic acid* *Right*	*Left*	*Decrease*
				mg. %		*%*
8	Normal	Saline	..	.....	452 ± 18	...
6	"	Epinephrine (1/250,000)	2	.....	294 ± 12	34.5
18	Hypox.	None	..	388 ± 13	377 ± 11	3.0
22	" †	Epinephrine (1/250,000)	2	386 ± 11	380 ± 10	1.3
8	" Incomplete	" "	2	398 ± 28	254 ± 18	36.2

* Rate: 0.5 ml. fluid per 100 g. body weight per hour.
† Experiment one day following 3-7 days' postoperative treatment either with ACTH or whole anterior pituitary.

ascorbic acid content of the second gland. Furthermore, it made no difference in the results whether the adrenal glands had been allowed to undergo atrophy following hypophysectomy, or whether they had been maintained by injections of ACTH, or by daily implants of fresh rat anterior lobe tissue.

It would appear, therefore, from these experiments that epinephrine administered either subcutaneously or intramuscularly does not act directly upon the adrenal cortex to produce an increased secretion, but can do so only by its capacity to stimulate the secretion of ACTH from the anterior lobe of the pituitary.

Endogenous epinephrine. The site of action of epinephrine released by the adrenals *in situ* was studied in experiments similar to those in which

TABLE 6

EFFECTS OF COLD, HEMORRHAGE, OR PAIN ON ADRENAL CHOLESTEROL AND ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS

No. of rats	*Procedure*	*Adrenal*		*No. of rats*	*Adrenal*	
		Cholesterol g. %	*Ascorbic acid mg. %*		*Cholesterol g. %*	*Ascorbic acid mg. %*
		Normal rats			*Hypox. rats**	
23	Controls	4.05 ± 0.14	423 ± 13	13	5.60 ± 0.43	418 ± 26
	Cold room, 0°-4° C.					
10	1 hr.	3.69 ± 0.74	233 ± 20	4	5.90 ± 0.32	379 ± 12
11	2 hr.	2.70 ± 0.19	290 ± 17	..	....	... ..
17	4 hr.	2.40 ± 0.17	... ..	8	5.72 ± 0.42	379 ± 18
18	6 hr.	2.07 ± 0.20	420 ± 35	..	....	... ..
9	24 hr.	2.19 ± 0.42	... ..	..	....	... ..
	Sensory nerve stimulation†					
6	End of stimulation	3.96 ± 0.38	270 ± 3	2	5.95	412 ..
6	30 min. after stimulation	4.16 ± 0.21	268 ± 4	..	....	... ..
6	60 min. after stimulation	3.08 ± 0.33	237 ± 18	..	....	... ..
	Hemorrhage‡					
6	1 hr.	....	166 ± 6	6	....	398 ± 24
6	3 hr.	....	251 ± 32	6	....	385 ± 23
6	6 hr.	....	292 ± 20	6	....	378 ± 26

* Three days postoperative. Fasting period shortened from 24 to 18 hours.

† Central end of sciatic nerve stimulated for 15 seconds every minute for fifteen minutes. Nembutal anesthesia.

‡ Rats bled 2% of body weight during one hour. No anesthesia.

epinephrine was given by injection. Long and Fry,[15] and Long[13, 14] have pointed out that the cholesterol and ascorbic acid of the adrenal gland decrease in amount following hemorrhage, scalding, cold exposure, or electrical stimulation of the central end of a mixed nerve, and they have noted that each of these conditions is known to bring about a release of epinephrine from the adrenal medulla. In order to determine whether this epinephrine may stimulate the adrenal cortex directly, or whether its action requires the intermediation of the anterior lobe of the pituitary, the experiments summarized in Table 6 were carried out on normal rats and rats

with the hypophysis removed. Normal animals exposed to a temperature of 0-4° C. showed a decrease in both the cholesterol and ascorbic acid content of the adrenals, the ascorbic acid falling the more quickly of the two (minimal level in one hour) and returning to the control level as the exposure was continued for 6 hours. The cholesterol fell more slowly (minimal level in 6 hours), and was only slightly restored even though the exposure was continued for 24 hours. These changes are dependent upon the anterior lobe, since in the experiments performed upon hypophysectomized rats, no significant change occurred in either the adrenal cholesterol or ascorbic acid content. Results of the same nature were obtained when hypophysectomized rats were subjected to stimulation of the central end of the sciatic nerve (under anesthesia), or to hemorrhage.

TABLE 7

EFFECT OF LAPAROTOMY AND REMOVAL OF ONE ADRENAL ON THE ASCORBIC ACID CONTENT OF THE SECOND ADRENAL* (NEMBUTAL ANESTHESIA)

No. of rats		*Adrenal ascorbic acid* *Right* mg. %	*Left* mg. %	*Decrease* %
12	Normal	406 ± 11	229 ± 6	43.6
18	Hypox.†	388 ± 13	377 ± 11	2.8

* Left gland removed one hour after right gland.

† Fasting period shortened from 24 to 18 hours.

The experiments recorded in Table 7 show that, in normal rats, the removal of one adrenal gland produces within one hour a marked decrease in the ascorbic acid content of the second gland. This fall does not occur in the absence of the pituitary, which suggests that it is brought about, as is the adrenal response to cold exposure, hemorrhage, or painful stimulation, through the release of epinephrine acting either directly or indirectly upon the anterior lobe. It seems clear from the results in the hypophysectomized animals that the action cannot be directly upon the adrenal cortex, and observations suggesting that the effect of epinephrine is a direct one upon the anterior hypophysis have already been published in preliminary form.[16] They will be further discussed in the second paper.

To the conditions already described, where the adrenal cortex becomes active presumably through the intermediation of epinephrine, there may be added another, viz., insulin hypoglycemia, already studied histologically by Marthe Vogt.[37] Utilizing normal rats and rats with denervated adrenals, Vogt found that under certain circumstances insulin brought about a loss both of adrenal cortical lipid and of medullary epinephrine. Rats with denervated adrenals did not show the loss of epinephrine, and many of

them also failed to undergo a loss of cortical lipid. Since some did lose cortical lipid, however, Vogt concluded that a secretion of epinephrine ". . . contributes to the loss of cortical lipids seen after insulin injection, but is not indispensable for it" (p. 403).

The data of Table 8 confirm Vogt's conclusion that insulin may activate the adrenal cortex via the adrenal medulla. Whether administered intraperitoneally (0.10 u./100 g.) or intravenously (0.05 u./100 g.), insulin caused a significant fall in adrenal ascorbic acid. In either case a hypoglycemia was found, with blood sugar levels of 25-30 mg. % at the end of

TABLE 8

EFFECT OF PRETREATMENT WITH GLUCOSE OR ADRENAL CORTICAL HORMONE ON THE ONE-HOUR RESPONSE OF THE NORMAL RAT ADRENAL TO INSULIN

No. of rats	*Injection*	*Units per 100 g.*	*Adrenal ascorbic acid mg. %*	*Blood glucose*		*Plasma amino N, 1 hour mg. %*
				Intial	*1 hour*	
				mg. %		
6	Saline	.....	381 ± 20	.....	73 ± 5	6.9 ± 0.3
7	Saline-Insulin	0.10 I.P.	207 ± 9	.....	28 ± 2	4.8 ± 0.2
7	Insulin	0.05 I.V.	273 ± 24	78 ± 3	26 ± 4	5.8 ± 0.2
6	Glucose*	.....	362 ± 14	.....	145 ± 4	5.5 ± 0.3
6	Glucose*-Insulin	0.10 I.P.	356 ± 30	.....	64 ± 10	5.4 ± 0.3
9	Insulin—Pretreated with cortical hormone†	0.10 I.P.	397 ± 19	.....	30 ± 2	4.4 ± 0.2

* Subcutaneous injection of 8 ml. of 10% glucose 15 minutes before the insulin.
† Rats received 2 ml. Upjohn's Lipo-Adrenal Extract 3 hours before the insulin.

one hour. In another group of experiments, glucose solution was injected before the insulin in order to prevent the hypoglycemia; under these conditions the fall in adrenal ascorbic acid did not occur, which shows that insulin as such is not an integral part of the activating mechanism. Since hypoglycemia brings into play most of the sympathetic nervous system, its influence upon the adrenal cortex is easily explained in the terms outlined above. Glucose may be said to block the mechanism in that it abolishes the hypoglycemia, which in this instance serves as the call for epinephrine secretion.

Inhibition of ACTH secretion. The account just given, however, is not the whole picture of the mechanism of ACTH secretion, as the last line of Table 8 demonstrates. Here the rats were treated with cortical extract, and then given insulin three hours later. As in the group pretreated with glucose, activation of the adrenal cortex was prevented by the cortical hormone. But there was an important difference between the two types of

pretreatment in that, whereas the glucose abolished the insulin hypoglycemia, the cortical extract did not do so. At the end of one hour the blood sugar level was 30 ± 2 mg. %. This hypoglycemia undoubtedly called forth a discharge of epinephrine; nevertheless, ACTH was not secreted, which means that the blocking action of the cortical hormone must have been different from that of the glucose.

The concept that cortical steroids may suppress ACTH release was adopted by Sayers and Sayers[34] to explain their finding that pretreatment with cortical extract prevents adrenal activation during cold exposure, heat, histamine, epinephrine, and the injection of typhoid organisms. We have, in part, confirmed their observations (see below), and in addition have attempted to learn the site of this blocking action—whether directly upon the anterior pituitary, or through some metabolic change associated

TABLE 9

EFFECT OF GLUCOSE PRETREATMENT ON THE ADRENAL ASCORBIC ACID OF NORMAL RATS EXPOSED TO COLD (1° C.) FOR ONE HOUR

No. of rats		*Adrenal ascorbic acid mg. %*	*Blood glucose† mg. %*
6	Fasted	234 ± 23	68 ± 3
7	Glucose injection*	234 ± 17	136 ± 11
6	Glucose—Room temp.	362 ± 14	145 ± 4

* Subcutaneous injection of 8 ml. of 10% glucose 15 minutes before exposure to cold.
† Samples taken at the end of cold exposure.

with the activity of cortical hormones or of epinephrine or of both. The well-known influence of the adrenal cortex upon carbohydrate metabolism (specifically, upon gluconeogenesis), suggested itself as the type of metabolic reaction which might be involved. If a need for carbohydrate were the critical factor, cortical steroids might increase the supply of carbohydrate and thereby inhibit the endocrine mechanisms concerned with gluconeogenesis. Experiments designed to test this hypothesis, however, failed to support it. As noted above, insulin hypoglycemia did not lead to increased adrenal cortical secretion when cortical extract had been given beforehand, in spite of the animal's need for extra glucose, as manifested in blood sugar levels of 30 ± 2 mg. %. Moreover, the administration of glucose to rats exposed to cold for one hour (Table 9) did not prevent cortical activation even though the animals had more than enough glucose for their metabolic needs and experienced slight hyperglycemia (136 ± 11 mg. %). In experiments where both cortical extract and epinephrine were given, the epinephrine retained its metabolic actions, including its effect upon muscle glycogen and blood lactic acid (Table 10). Nevertheless, the

adrenal ascorbic acid of the group treated with cortical steroids fell only 72 ± 16 mg. % in response to epinephrine, while that of the rats not pretreated fell 218 ± 16 mg. %. All of these experiments suggest a direct inhibition or blocking of the anterior pituitary by the cortical hormones.

Our experiments did not confirm those of Sayers in one respect, that is, that desoxycorticosterone acetate also exerts a blocking action. Cheng and

TABLE 10

THE INFLUENCE OF ADRENAL CORTICAL HORMONE PRETREATMENT UPON THE METABOLIC EFFECTS OF EPINEPHRINE IN THE NORMAL RAT

	*Epinephrine**	*Epinephrine plus cortical extract*†*
Number of rats	9	10
Muscle glycogen, mg. %		
Initial	549 ± 11	568 ± 20
Loss	156 ± 24	177 ± 17
Liver glycogen, mg. %		
Final	215 ± 4	512 ± 7
Blood glucose, mg. %		
Initial	66 ± 3	68 ± 3
Gain	34 ± 8	63 ± 9
Blood lactic acid, mg. %		
Initial	13 ± 1	13 ± 2
Gain	13 ± 2	11 ± 2
Adrenal ascorbic acid, mg. %		
Initial	422 ± 19	450 ± 9
Loss	218 ± 16	72 ± 16

* All rats were fasted 24 hours before epinephrine (0.02 mg./100 g.) was injected subcutaneously. They were sacrificed one hour later.

† Two ml. of Upjohn's Lipo-Adrenal Extract was injected 1½ to 3 hours before epinephrine injection.

Sayers[4] have shown that large doses of D.C.A. implanted as pellets lead to an increased insulin sensitivity in demedullated rats. This hypersensitivity they attributed to cortical insufficiency, an expression of an inhibition of the anterior pituitary. Their report, however, did not mention any direct measurement of adrenal function (such as determination of adrenal ascorbic acid or cholesterol, or changes in the various white blood cell counts), Nor did they consider the possibility that some complication, possibly a toxic effect of the large doses of D.C.A. or a diminished food intake, may have contributed to their results. In our experience, as shown in Table 11, whether given one or three hours before cold exposure, neither D.C.A. in

oil nor D.C.A. in suspension prevented the fall in ascorbic acid. By contrast, the lipoadrenal extract completely prevented the change in ascorbic acid when given three hours prior to cold exposure, and allowed only an insignificant fall when given one hour before the rats were placed in the cold room. These data indicate that, at least in these doses in acute experiments, the synthetic compound D.C.A. does not block ACTH secretion. In view of the rather striking actions of D.C.A. upon electrolyte metabolism, these data further suggest that electrolyte changes are not an important part of the mechanism stimulating ACTH output.

These experiments also suggest that a certain length of time may be needed for the blocking action to occur, since the blocking was greater when the cortical steroids were given four hours than when injected only one

TABLE 11

EFFECT OF PRETREATMENT WITH D.C.A. OR LIPO-ADRENAL EXTRACT ON ADRENAL ASCORBIC ACID OF RATS EXPOSED TO COLD (0°-4° C.) FOR ONE HOUR

No. of rats	*Procedure*	*Time between injection and exposure hrs.*	*Adrenal ascorbic acid mg. %*
18	Controls	..	427 ± 8
6	Cold exposure	..	237 ± 13
4	" " + Lipo ext. 2 ml.	1	385 ± 20
3	" " " " " "	3	431
5	" " " D.C.A. in oil 4 mg.	1	226 ± 26
6	" " " " " " "	3	319 ± 32
3	" " " " suspension "	1	250
3	" " " " " "	3	209

hour before cold exposure. Furthermore, the blocking action of a given amount of cortical extract may be greater when an animal is subjected to a relatively light stress than when the stress is more severe (unpublished data). There appear to be definite quantitative relationships between the severity of a stress, the amount of cortical extract needed to suppress ACTH secretion, and the time intervals during which the stress and the injected steroids are acting.[25]

The specific nature of the blocking of ACTH release by injected cortical extract is also apparent in experiments where other types of blocking agents have been found to be without effect upon the hypophysis, as Tepperman and Tepperman[32] have noted. The data of Table 12 confirm their report, showing that ergotamine tartrate, an autonomic blocking agent, does not alter the adrenal cortical response to epinephrine; rather, ergotamine activates the cortex when given by itself. Results similar to these have also been obtained with other blocking agents, including dibenamine and tetra-

ethylammonium chloride[8, 30] (Fry, unpublished), and it appears likely that drugs of this type have as one of their actions a preliminary excitation of the central nervous system and adrenal medulla.[19]

ACTH secretion in the absence of epinephrine. Vogt's[37] studies following denervation of the adrenals have already been referred to, with her conclusion that the medulla is involved in but not indispensable to cortical activation. Her data were sometimes equivocal on this point, possibly because it is difficult to denervate the adrenal without injuring its blood supply. Attempts to repeat this work have not given entirely satisfactory results in our hands. Since the point is an important one, however, another type of experiment was performed for the same purpose, that is, to attempt

TABLE 12

FAILURE OF ERGOTAMINE TARTRATE TO PREVENT THE EFFECT OF EPINEPHRINE ON ADRENAL CHOLESTEROL AND ASCORBIC ACID OF THE NORMAL RAT

No. of rats	*Injection*	*Adrenal*	
		Cholesterol g. %	*Ascorbic acid mg. %*
11	Epinephrine*	2.26 ± 0.22	216 ± 9
6	Ergotamine†-Epinephrine*	2.94 ± 0.32	248 ± 10
7	Ergotamine†	....	267 ± 26

* Subcutaneous injection of 0.02 mg./100 g. body weight each hour for 4 hours.
† Subcutaneous injection of 0.6 mg./100 g. body weight 15 minutes before the first injection of epinephrine.

to assess the importance of epinephrine in stimulating a secretion of ACTH. To this end the adrenal medullae were removed from a series of rats to deprive them of their endogenous epinephrine. Following demedullation, a definite sequence of changes occurs in the adrenal remnants, resulting in regeneration of cortical tissue capable of maintaining the rat without evidence of cortical deficiency.[11] The ascorbic acid concentration remains low (Table 13, line 2), but it is decreased still further when ACTH is given (Fry, unpublished data).

Experiments carried out upon these animals showed that the adrenal medulla is sometimes required, sometimes not required for increased cortical activity. Stimulation of an afferent nerve trunk for 15 minutes in demedullated rats did not alter the adrenal ascorbic acid concentration (Table 13), whereas in normal rats, a marked fall had been found under similar conditions (Table 6). This difference in response suggests that the cortical stimulation seen in the normal animals was brought about through a reflex secretion of epinephrine, and emphasizes the fact that the mechanism is a rapidly acting one since it can produce the change within a relatively short time. On the other hand, the cortex can still be stimulated

in the absence of the medulla (although, as will be shown in the later paper, the stimulation is delayed). Thus, fasted, demedullated rats showed a very real degree of cortical activation when exposed to cold for 4 hours, or 3-4 hours after removal within one hour of blood amounting to two per cent of their body weight. Under these latter conditions epinephrine is not required and the secretion of increased amounts of ACTH can occur in the absence of the adrenal medulla.

It will be apparent that there must be at least two mechanisms influencing the anterior lobe in its adrenotrophic function. One is a quickly acting mechanism operating through the adrenal medulla and release of epinephrine; the other is slower and remains after the medulla has been removed. The nature of these two mechanisms and their interaction are described in detail in the paper which follows.

TABLE 13
EFFECT OF ADRENAL DEMEDULLATION ON RESPONSE OF ADRENAL CHOLESTEROL AND ASCORBIC ACID TO COLD, HEMORRHAGE, AND PAIN

No. of rats		*Body weight g.*	*Adrenal*		
			Weight g./100 g. B.W.	*Cholesterol g. %*	*Ascorbic acid mg. %*
23	Normal controls	235 ± 4	14.0 ± 0.1	4.05 ± 0.14	423 ± 13
17	Adrenal demedullated controls	212 ± 7	12.1 ± 0.6	2.48 ± 0.15	237 ± 16
3	Sciatic stimulation†	225 ± 13	9.4 ± 0.3	2.26 ± 0.35	241 ± 48
6	Cold (4° C., 4 hrs.)	188 ± 2	11.9 ± 0.7	1.50 ± 0.18	129 ± 12
7	Hemorrhage*	235 ± 7	15.0 ± 0.7	1.52 ± 0.23	98 ± 11

* Blood loss in one hour equivalent to 2 per cent of body weight. Adrenals removed 3-4 hours later.

† Central end of sciatic nerve stimulated for 15 seconds every minute for 15 minutes. Adrenals removed immediately following stimulation.

Discussion

It is now possible to account for some of the apparently contradictory results earlier obtained in studying pituitary-adrenal relationships. Since there appear to be at least two mechanisms by which ACTH secretion may be stimulated, it is not surprising that experiments designed to study only one sometimes gave equivocal results. We have encountered this difficulty often, as did Vogt[37] in her study of the action of insulin upon the adrenal cortex. It seems likely that the cortical activation which she noted in 3-4½-hour experiments upon animals with denervated adrenals may have been brought about through the slower of the two types of mechanisms, the "metabolic phase" of McDermott, *et al.*[17] Long's question[14] as to whether activation of the adrenal cortex "depends on" a release of epinephrine may be answered in similar terms. As the data of Table 13 indicate, under

certain conditions epinephrine is required, especially when rapid cortical activation is to be brought about in response to mild stimuli. If the disturbance to which the animal is subjected is severe or prolonged, however, cortical activation occurs without epinephrine. Another question which may be answered concerns the sensitivity to stress of animals with demedullated or denervated adrenal glands.[14] Such animals, though more sensitive than normal rats,[88] are much more resistant than adrenalectomized or hypophysectomized animals, because secretion of ACTH and cortical hormones can still occur even when epinephrine secretion has been prevented.

Another source of confusion has been a failure to realize that the timing of experiments is a critical matter. McDermott, *et al.*,[17] have found that responses to endogenous epinephrine are best studied within one hour, since by the end of four hours, the "metabolic phase" may intervene to obscure the original response. The latter reaction may have been a factor in Vogt's insulin experiments, which were continued for as long as four and one-half hours.[27] Many of the earlier experiments done in various laboratories were carried out under this handicap, although when reviewed with this point in mind, they may acquire a meaning at first not apparent. Finally, the ease with which animals release their own epinephrine has caused a certain amount of difficulty. It is true that by careful handling of the animals, warming of solutions to be injected, and appropriate use of anesthesia when indicated, the effects of fright and pain can be minimized.[23] But care and experience are required to accomplish this result; and where the usual laboratory routines are followed, autonomic activity almost certainly occurs in the early stages of most experiments. A striking example of the effect of endogenous epinephrine is provided by the experiments where laparotomy was done for removal of one adrenal, followed by removal of the second gland one hour later (Table 7). During and after removal of the first adrenal, the spinal cord must have received from the abdomen and viscera a bombardment of sensory impulses which reflexly stimulated the medulla of the remaining gland, and thus activated the anterior pituitary and the adrenal cortex.

It may be well to point out the unusual sensitivity of this mechanism, and the small quantity of epinephrine necessary to set it in action. Given intramuscularly, a quantity as small as 0.04 μg./kg./min. (0.290 μg./100 g./hr.) was effective when administered for 60 minutes, while 0.08 μg./kg./min. was adequate when given for half that time, or when infused intraperitoneally for one hour. Results following intravenous infusion were probably like these, but they are more difficult to interpret because control studies with intravenous infusion of saline showed that endogenous epinephrine was being released. The experiments using intraperitoneal or intramuscular infusions were more conclusive because saline given by these routes was without effect. The small doses of epinephrine used here may be compared with those used by Cori, Cori, and Buchwald,[5] who found that in

anesthetized rabbits 0.5 μg./kg./min. had only a slight effect on blood pressure; in the absence of anesthesia, 0.05 μg./kg./min. had no hyperglycemic action, and 0.1 μg./kg./min., infused for two hours, produced mild hyperglycemia. The doses which we have used compare favorably with these, and appear to be well within the physiological range. The data of Table 4 (above) indicate that epinephrine may stimulate the anterior pituitary when infused in amounts too small to cause a rise in blood sugar. Unpublished data show that not only blood sugar, but also serum potassium concentration, lactic acid, and amino acids as well as muscle glycogen may remain unaffected by small doses of epinephrine which do, nevertheless, increase ACTH secretion (Fry). On the other hand, as noted earlier (Table 10), after the anterior lobe has been blocked by the administration of cortical steroids, larger doses of epinephrine fail to stimulate the gland although they have their usual metabolic effects. These experiments seem to indicate that the metabolic effects of epinephrine are not correlated with its action upon the anterior pituitary gland.

That epinephrine is an agent capable of exciting the adrenal cortex is now generally accepted,[15, 22, 25, 34, 37] and does not need further emphasis. How this excitation is brought about has been a matter of debate.[20] Unlike the earlier experiments of Vogt,[36] our data seem to show without exception that ACTH is the intermediary of the epinephrine activity, and that the site of action of the epinephrine is the anterior lobe. This finding is in accord with the data of Pincus and Hechter, who were unable to detect any increased output of steroids from perfused beef adrenals when epinephrine was added to the perfusate.[20] This conclusion also agrees with that of McDermott, Fry, Brobeck, and Long,[16] who by applying epinephrine directly to anterior lobe tissue were able to demonstrate a direct activation. Their data indicate that epinephrine cannot be said to have this action through any influence that it may have upon the rate of peripheral utilization of cortical hormone, as proposed by Sayers.[25] This subject will be considered further in the following paper.

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MECHANISM OF CONTROL OF ADRENOCORTICOTROPHIC HORMONE*

W. V. McDERMOTT,† E. G. FRY, J. R. BROBECK, AND C. N. H. LONG

Experiments indicating that epinephrine activates the adrenal cortex only when the pituitary gland is intact[12, 18] have led to the suggestion that epinephrine may exert an action directly upon the secretory cells of the anterior hypophysis.[18] At the same time, a number of other drugs, notably histamine,[21] have also been shown to bring about the release of ACTH, and this finding has tended to relegate epinephrine to the role of a nonspecific agent in the activation mechanism. This is true particularly since a series of reports published by Sayers and his associates[8, 21] have demonstrated that the output of adrenocorticotrophic hormone is suppressed when the level of adrenal cortical hormones rises in the circulating blood—an observation which has suggested that the level of circulating cortical hormones controls the ACTH output of the pituitary in much the same manner that the level of estrogens influences the output of the follicle stimulating hormone. It may be noted, however, that histamine as well as many other drugs which stimulate the release of ACTH are known to cause also a rapid release of epinephrine from the adrenal medulla,[16] so that the possibility remains that a variety of other drugs may exert their action through the epinephrine mechanism.

Since stating the original hypothesis concerning the role of epinephrine in activating the pituitary-adrenal system,[12] Long and his associates have continued to study the specificity of the response and the mechanisms through which it is brought about. Their data appear to show that epinephrine does not activate the pituitary through any of its other metabolic actions on electrolyte or carbohydrate metabolism. While they confirm, in part, the work of Sayers and his colleagues, the data of Long, *et al.*,[18] and of McDermott, *et al.*,[15] suggest that epinephrine has a specific role and an action directly upon the pituitary gland.

In order to attempt a correlation of the data available on the mechanisms controlling the secretion of ACTH, and to investigate further the role of epinephrine in this control, it seemed necessary to compare normal animals with animals which were unable to liberate endogenous epinephrine in

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† Fellow of the United States Public Health Service, National Institute of Health. Present address, Massachusetts General Hospital, Boston.

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response to painful, noxious, or emotional stimuli. Preliminary experiments of this type utilizing demedullated rats are described in the preceding report. To extend these studies in small animals, the most practical methods appeared to be the enucleation (demedullation) of the adrenal glands which was mentioned earlier, transection of the spinal cord above or below the splanchnic outflow, and the placing of electrolytic lesions in the diencephalon (the latter suggested by earlier studies indicating that increased activity of the sympathetic nervous system and the subsequent release of epinephrine are mediated through the thalamus and hypothalamus[1, 9, 10]).

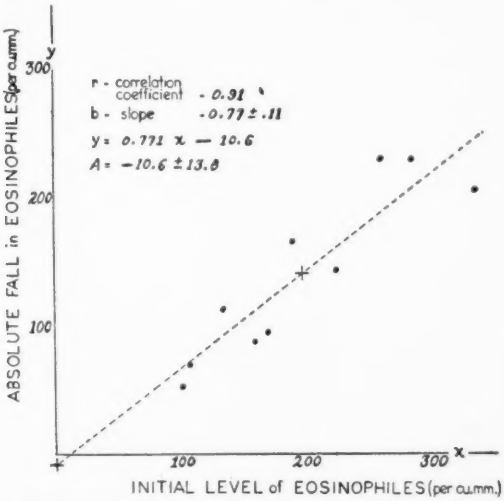


FIG. 1. Correlation between initial level of eosinophiles and absolute fall in a comparable group of normal animals injected subcutaneously with epinephrine, 0.02 mg./100 g.

Methods

An account of the methods utilized has been given in the preceding report. All experiments described below were done upon male albino rats of the Sprague-Dawley strain, and none of the rats was fasted before being used for an experiment.

In nearly all of the experiments which follow, the direct counting of eosinophiles in the peripheral blood[17] was used as an index of the rate of secretion of adrenal cortical hormone.[18] Because of variations in initial levels of eosinophiles, it was decided to chart the results graphically by percentage fall. To obviate criticism of the use of a percentage rather than an absolute change, a graph (Fig. 1) has been included which demonstrates a high correlation coefficient between the initial levels and the absolute fall in eosinophiles, a relation which makes it statistically permissible to employ percentage fall as an accurate index of change. In order to avoid basing all conclusions on eosinopenic responses, a number of animals were sacrificed during a variety of

experiments and at various stages of the experiments, and their adrenal glands were analyzed chemically. Figure 2 is a scatter diagram illustrating the correlation found between changes in eosinophiles in the peripheral blood and changes in adrenal ascorbic acid concentration in normal rats subjected to procedures known to activate the adrenal cortex. The high correlation coefficient and the slope of the two regression curves indicate that the eosinopenic response is at least as satisfactory an index of adrenal cortical secretion as the fall in ascorbic acid content of the adrenal. The eosinophile count has the added advantage that changes can be observed at various times in an experiment on a single animal.

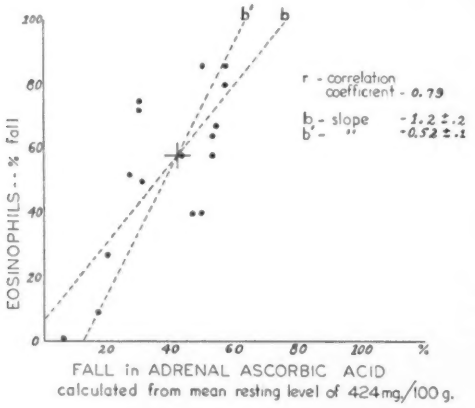


FIG. 2. Correlation between ascorbic acid levels in the adrenal glands and per cent fall in eosinophiles in a series of normal animals studied at varying time intervals, with epinephrine and under the various conditions of stress utilized in these investigations.

Results

It was decided to select as a pilot experiment a comparison of the changes in eosinophiles which follow the subcutaneous administration of epinephrine, using six normal and six spinal rats. The results are shown graphically in Figure 3. These curves, illustrating an initial rise followed by a fall, are in general quite similar to those described by Rud,[10] Godlowski,[3] and Hortling.[8] The curves have the same contour in normal and spinal rats, although a marked time lag may be noted in the latter group. Since the spinal sections were made in an attempt to interrupt pathways responsible for the secretion of epinephrine, these results suggest that the more rapid reaction of the normal rats was due to endogenous epinephrine released intravenously in response to the somewhat painful injection, plus the emotional stress of handling, cutting the tail, etc. In comparing these curves, it is clear that significant conclusions might be drawn by making eosinophile counts at zero, one-, and four-hour intervals, since the one-hour interval appears

to represent the most marked point of difference between the normal animal and the animal which fails to respond to mild stress with a secretion of endogenous epinephrine, while the four-hour interval apparently represents in all animals a close-to-maximal response to a given drug or stimulus. Therefore, these particular time intervals were selected for further experiments.

As an incidental finding, attention might be called to the effect of splenectomy on the response of the eosinophiles to epinephrine.[6, 18] It would appear from the results shown in Figure 3 that, in the rat at least, the initial rise of the eosinophiles in response to exogenous or endogenous epinephrine

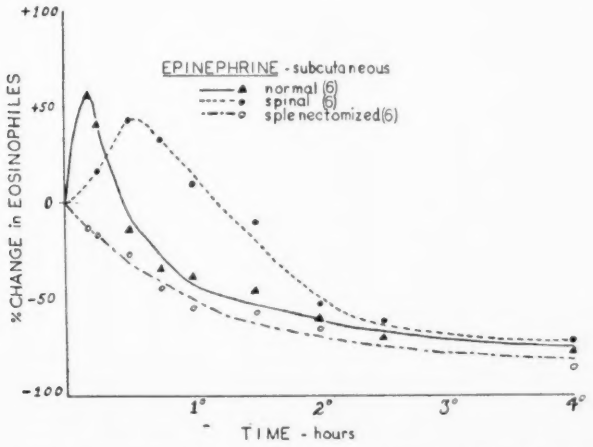


FIG. 3. Curves showing the per cent change in eosinophiles at specific time intervals after subcutaneous injection of epinephrine (0.02 mg./100 g.) in normal, spinal, and splenectomized rats.

is due to a contraction of the spleen, since this rise is abolished by splenectomy. The remaining experiments were done upon rats with the spleen intact, since the initial rise proved to be important in emphasizing the differences between the various categories of animals.

Saline. As a control series, various types of animals were injected subcutaneously with 2.0 ml. of normal saline at room temperature. From the results charted in Figure 4, it is apparent that only the normal animals demonstrated any eosinopenic response, and that their response was maximal in one hour.* A comparison of the curves in Figure 3 with those of later experiments done with epinephrine (Figs. 5, 6, and 7) suggests

* Dots above the columns in all charts represent the calculated standard error.

that the response to isotonic saline represents a reaction of the sympathetic nervous system to a minimal stress, with subsequent release of epinephrine. As noted in the preceding paper, this response can be almost abolished by the careful selection of animals which are used to handling, by warming the saline, and by gentle injection.

Epinephrine. The next obvious step was a comparison of the effects of subcutaneous epinephrine in normal rats and in rats in which the pituitary-adrenal axis had been broken by ablation of either the pituitary or the adrenal gland. In agreement with the results of earlier experiments using

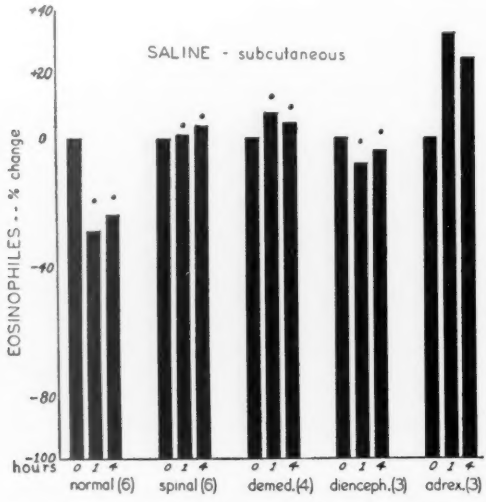


FIG. 4. Eosinophile response following the subcutaneous injection of isotonic saline in normal, spinal, adrenal demedullated, and diencephalic rats.

adrenal ascorbic acid levels as an index of cortical activity,[12, 13] the eosinopenic response did not occur in the hypophysectomized rats (Fig. 5), although they did show the usual preliminary rise in eosinophiles following administration of epinephrine. Adrenalectomized rats were expected to react in an identical fashion; they showed, however, a slight but statistically significant eosinopenic response. In an attempt to interpret this unexpected result, a series of rats was used in which the testicles were also removed at the time of the adrenalectomy. The results of epinephrine injected subcutaneously in these rats paralleled those obtained in hypophysectomized rats. This suggests that there may be a slight overlap in the effects of the adrenal and gonadal steroids, or that there may be adrenal rests in the testes which

are responsive to the action of ACTH, but further investigation of this phenomenon is not within the bounds of this report.

It having been established that there is no eosinopenic response to epinephrine in animals in which the pituitary-adrenal axis is interrupted, the effects of subcutaneous epinephrine are now to be compared in normal rats and in rats in which the release of endogenous epinephrine has been abolished (i.e., spinal, adrenal demedullated, and diencephalic animals). This comparison demonstrates that, while the response in four hours is quite similar, there is in the one-hour reaction a distinct difference between normal rats and the rats which fail to liberate endogenous epinephrine (Fig. 6).

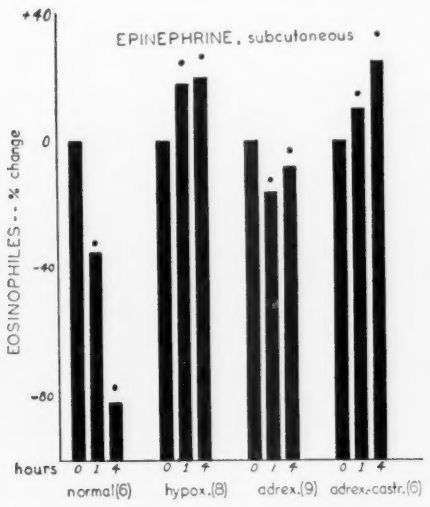


FIG. 5. Eosinophile response following the subcutaneous injection of epinephrine (0.02 mg./100 g.) in normal, hypophysectomized, adrenalectomized, and adrenalectomized-castrate rats.

If this difference is due to the epinephrine released from the adrenal medullae of the normal rats in response to the injection, it should be possible by the intravenous injection of epinephrine to reproduce in spinal and adrenal demedullated rats the more rapid type of fall shown by the normal animals. The results of intravenous (femoral vein) administration of epinephrine in spinal and adrenal demedullated rats are charted in Figure 7 and demonstrate that the one-hour effect is reproducible. The final series of blocks in Figure 7 illustrates that, by simultaneous intravenous and subcutaneous administration of epinephrine, one can evoke in spinal rats an eosinopenic response very similar to the response of the normal rat to a single subcutaneous injection.

Cold exposure. The response of the eosinophiles of non-fasting, normal rats exposed to cold (0-2° C.) for four hours showed an initial fall in one hour with no further drop (Fig. 8). The similarity of these results and those obtained from a single injection of isotonic saline (Fig. 4) suggests that the normal rat responds to this degree of cold exposure with an initial sympathetic discharge, and then adapts well to the new environment. Theoretically, therefore, animals whose ability to release epinephrine has

been abolished should show no significant change in eosinophile count under similar conditions. The results of exposure to cold (0-2° C. for four hours) in non-fasted, adrenal demedullated and diencephalic rats bear out this hypothesis (Fig. 8). (Cf. with the response of fasted, demedullated rats of Long, *et al.*[18]) Spinal rats, however, although unreactive in one hour, showed a marked drop in eosinophiles in four hours, and a number of animals were almost moribund at the end of this period of exposure. This amount of cold, while it did not provide any marked degree of stress to the other categories of rats, appeared to be an extreme physiological stress to the spinal animals, whose normal protective mechanisms against cold exposure had been almost abolished by transection of the cord at the third thoracic vertebra.

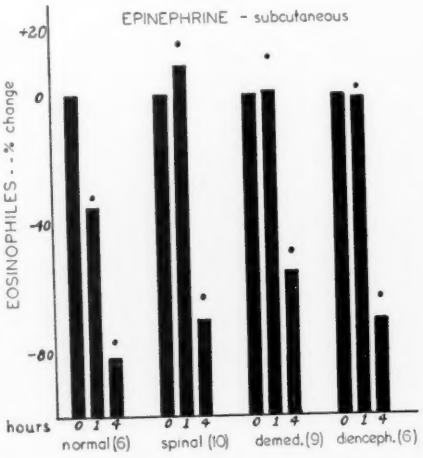


FIG. 6. Eosinophile response following the subcutaneous injection of epinephrine (0.02 mg./100 g.) in normal, spinal, adrenal demedullated, and diencephalic rats.

Obviously, the release of epinephrine is not the only mechanism controlling the secretion of adrenocorticotrophic hormone from the pituitary, since some other explanation is necessary for the four-hour response of the spinal rats exposed to cold (cf. also Long, *et al.*[18] and Recant, *et al.*[18]). Of the various explanations which might be offered, the most plausible one seems to be the hypothesis of Sayers and Sayers[21] that an increased utilization of adrenal cortical hormone (ACH) under any severe stress, with a resultant fall in the level of circulating ACH, provides a stimulus for release of adrenocorticotrophic hormone. If this hypothesis is applicable in this instance, by providing a severe stress it should be possible to cause a four-hour fall in eosinophiles in both the adrenal demedullated and in the diencephalic rats, in spite of their inability to liberate epinephrine.

Laparotomy. As an extreme stress, it was decided to open the peritoneal cavity under Nembutal anesthesia (5 mg./100 g., intraperitoneally), to expose the intestines on the abdominal wall for three minutes, and then to close the abdomen. The results of these investigations are charted in Figure 9, where they demonstrate that, while there is no eosinopenic response in one hour in the adrenal demedullated and diencephalic rats, at

the end of four hours there is a dramatic fall comparable to that obtained in normal rats. This supports the impression that there are other factors in addition to epinephrine involved in the release of ACTH; the implications of these experiments will be discussed later.

Pain stimulus above the spinal cord transection. At this point, it seemed important to determine whether or not a painful stimulus which entailed no physiological stress could bring about a release of ACTH via neural or neurohormonal pathways in the absence of epinephrine release. The subcutaneous injection of 1 ml. of hypertonic (10%) saline proved to be a satisfactory pain stimulus lasting several minutes in the normal rat, consistently causing a definite eosinopenic response in the first hour without further fall. The injection caused no real injury to the animals. A series of six spinal rats was then treated in a similar fashion. In both the normal controls and in the spinal animals, the injections were made beneath the skin of the scalp (well above the level of the spinal cord transection at the third dorsal vertebra). The results (shown in Fig. 10) demonstrate that, even though sensory pathways from the site of the pain stimulus to the higher centers are intact, there is in spinal rats no eosinopenic response such as occurred in the normal controls. Presumably, therefore, the release of ACTH which occurs following a painful or emotional stimulus (in the absence of severe physiological stress) is dependent on the reflex secretion of epinephrine and is not mediated through any other neural or neuro-humoral pathway. There appears to be no mechanism within the cervical cord, brainstem, hypothalamus, or pituitary stalk which is capable of activating the anterior pituitary under the conditions of this experiment, when epinephrine secretion is abolished.

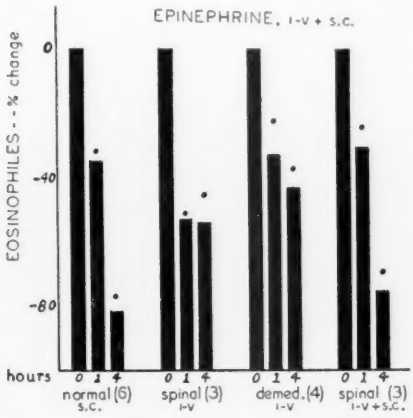


FIG. 7. Eosinophile response following the injection of epinephrine subcutaneously (0.02 mg./100 g.) in normal rats, intravenously (0.002 mg./100 g.) in spinal and adrenal demedullated rats, and intravenously and subcutaneously (same dosage) in spinal rats.

Site of action of epinephrine. As a final experiment in this series, it was decided to attempt to localize the site of action of epinephrine by the use of hypophysectomized animals with anterior pituitary tissue transplanted into the anterior chamber of the eye. All of the data thus far obtained in this laboratory had indicated that, while the epinephrine mechanism is not the

only one acting upon the anterior lobe, the adrenal medulla appears to be the more rapidly acting and the more highly sensitive of the mechanisms thus far proposed. From indirect evidence reported in the preceding paper, showing that there is no good correlation between the actions of epinephrine upon the pituitary and upon other organs of the body (for example, skeletal muscle), the locus of action of the hormone of the adrenal medulla in releasing ACTH was concluded to be directly upon cells of the anterior hypophysis.

It has not been possible to test this conclusion by direct application of epinephrine to the pituitary *in situ* because every operation thus far attempted to expose the pituitary in normal animals has brought about a marked activation of the adrenal cortex, presumably autonomic in origin. Nor has it been possible with any certainty to rule out the complicating features of some "metabolic" type of change in order to justify the use of demedullated, spinal, or hypothalamic animals in such experiments. It seemed desirable, therefore, to place the pituitary in a more accessible position; this was done by the method of Greene (as described in the preceding report) a method similar to those employed by Martinovitch,[14] Cheng, *et al.*,[5] Fortier and Selye,[7] and Schweizer and Long.[22]

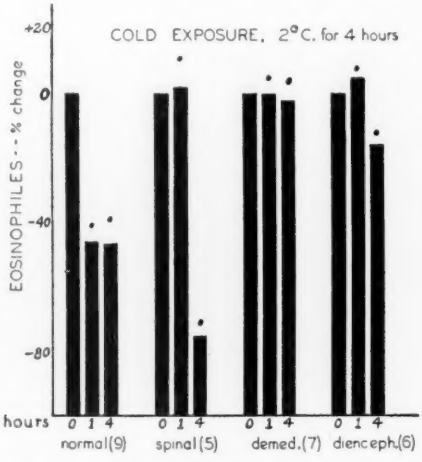
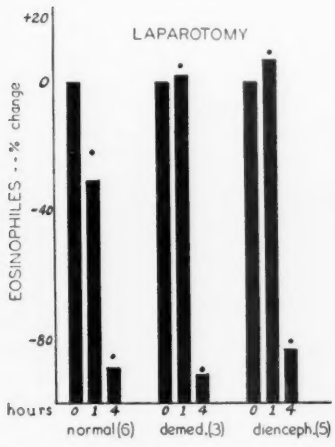


FIG. 8. Eosinophile response during exposure to cold (2° C.) for four hours in normal, spinal, adrenal demedullated, and diencephalic rats.

Six weeks after the grafting operation, tests for adrenocorticotrophic function were begun on the seven animals successfully prepared. The data of Table 1 show that animals with engrafted anterior pituitaries responded to a painful injection (10% saline) like normal rats, with falls of 19-61% in eosinophile counts. Epinephrine injected subcutaneously had a similar but more uniform effect, producing falls of from 34% to 61%, while the injection of 0.05 ml. of epinephrine solution directly into the anterior chamber where the graft was located produced an eosinopenia of —34% to —52%. On the other hand, the injection of the same amount of epinephrine into the opposite or "normal" eye evoked no significant fall in

the eosinophile count,* while subcutaneously injected epinephrine produced no fall after surgical removal of the eye bearing the graft (0 to 25% rise in the count).

From these data one may conclude that epinephrine has the direct type of action proposed above. Given subcutaneously in the usual dosage (0.02 mg./100 g.) it was borne to the engrafted gland where it stimulated the secretion of ACTH. Placed into the anterior chamber in a much smaller dose (0.2 μg.) its action was the same if the eye contained the transplant; if the opposite eye was used, only insignificant changes occurred, since the



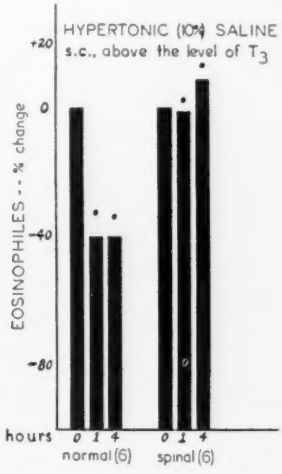


FIG. 9. Eosinophile response following laparotomy in normal, adrenal demedullated, and diencephalic rats.

FIG. 10. Eosinophile response following subcutaneous injection (1.0 ml.) of hypertonic (10%) saline above the level of spinal cord transection (T_3) in spinal and normal rats.

epinephrine reached the pituitary after having been much reduced in concentration while traversing the general circulation.

These results confirm those of Cheng, *et al.*,[4,5] and of Fortier and Selye,[7] who found direct pathways between the pituitary and hypothalamus to be unnecessary for adrenocorticotrophic function of the anterior lobe. The engrafted pituitaries in our series did not completely repair the deficiencies of the hypophysectomized animals in that growth did not occur, the testes remained atrophic during the time of this study, and assays for gonadotrophic activity in the transplants were negative. The adrenal glands, how-

* In some of the rats the eye bearing the graft was tested first, while in other rats the injection was made first into the normal eye. Results were the same in both cases.

ever, were larger than in control hypophysectomized rats, the grafts were found by assay to have adrenocorticotrophic activity (cf. McDermott, *et al.*[15]), and there was no question about the eosinophile response to pain or to epinephrine.* That these responses were brought about by the graft and not by some other mechanism, nor by tissue which might have remained in the sella, is shown by the failure of response after extirpation of the engrafted eye.

Discussion

The results of these experiments demonstrate that the activation of the autonomic nervous system with a consequent release of epinephrine plays an important but by no means a solitary role in the mechanism controlling the release of adrenocorticotrophic hormone from the pituitary. On the basis of these investigations and the results reported by Ingle,[11] Sayers,[21] Cheng,[8] and Recant,[18] and their respective associates, there appears to be a dual mechanism controlling the release of ACTH, one phase of which depends on the activation of the sympathetic nervous system, and the other on the utilization of adrenal cortical hormones (ACH) in the body. We have illustrated diagrammatically in Figure 11 our concept of this dual mechanism and have labelled the two phases as (i) *autonomic* and (ii) *metabolic*. In the normal organism exposed to severe stress, these phases appear to be sequential, with a rapid activation of the anterior pituitary through the release of epinephrine, followed by the metabolic phase in which the bodily requirements for the adrenal cortical steroids control the release of ACTH through the medium of the varying levels of adrenal cortical hormones in the circulating blood (Sayers' hypothesis[21]). That these two phases are not necessarily sequential or concomitant even in the normal animal is indicated by the data in Figures 4 and 8, in which the mild, transient stimulus of an injection, or the initial stress followed by adaptation which occurs during exposure to cold at 0-2° C., is shown to cause in one hour an initial eosinopenic response followed by stabilization. In the spinal, adrenal demedullated, and diencephalic animals it is apparently only the metabolic phase which functions, and unless the stress is severe enough to cause an increased utilization of adrenal hormone, no eosinopenic response will occur.

The role of the diencephalon in the release of adrenocorticotrophic hormone appears to be as a center in the long neural pathway effecting the release of epinephrine. It appears likely that the results of Recant, *et al.*,[18] who found that Nembutal acts to stabilize the eosinophile count, and those of Sayers and Sayers,[21] who noted that barbiturate anesthesia prevents the adrenal cortical response to cold, may be explained by the well-known selective action of barbiturates upon the hypothalamus. Any drug which acts to depress the autonomic function of the hypothalamus, or specifically,

* The ability of grafts of this type to repair the abnormalities which follow hypophysectomy is being further studied.

its "sympathetic" function, would be expected to modify the autonomic phase of ACTH release.* From the data available, no specific neuroanatomical conclusions can be drawn beyond the fact that the electrolytic lesions effective in abolishing the endogenous secretion of epinephrine as a response to stress were located in the ventral thalamus and anterior

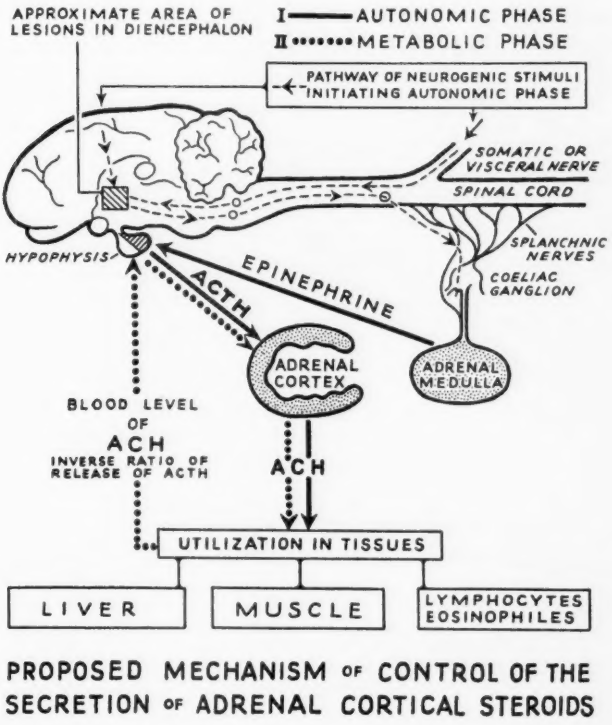


FIG. 11. Diagrammatic representation of the proposed mechanism of control of the secretion of adrenal cortical hormones.

hypothalamus. From histological study no specific nuclei or cell groups can be implicated, and it is our impression that some of the results may have been achieved by interruption of afferent or efferent tracts to or from the hypothalamus. In no case was it possible to abolish the metabolic phase of the release of ACTH, even with very large lesions which destroyed not

* With procedures such as laparotomy or stimulation of a sensory nerve, responses may be obtained even though the rats have been given an anesthetic dose of Nembutal, although it appears probable that under deeper anesthesia the autonomic phase might be completely inhibited.

only the paraventricular nuclei, but almost the entire anterior hypothalamus. As mentioned before, only six diencephalic animals are included in this report, and further investigations are in progress. From the results of these studies, including those on pain stimuli as charted in Figure 10, it would appear that no neural or neurohormonal pathways are concerned specifically with the release of ACTH, beyond the well-established pathways of the sympathetic nervous system which effect the release of epinephrine. From the adrenal medullae the epinephrine appears to be carried to the anterior lobe where it acts directly upon secretory cells to stimulate a release of adrenocorticotrophic hormone.

TABLE 1

EOSINOPHILE RESPONSE IN HYPOPHYSECTOMIZED RATS WITH PITUITARY GRAFTS

	Percentage change in eosinophiles							
Procedure	*Rat no. 1*	*2*	*4*	*5*	*7*	*8*	*9*	*Av.*
NaCl, subcutaneously 1 ml. of 10% sol.	—32	—39	—54	—61	—44	—30	—19	—40 ± 5
Epinephrine, subcutaneously 0.02 mg./100 g.								
Before removal of grafted eye	—61	—49	—49	—34	—46	—41	—41	—46 ± 3
After removal of grafted eye	+ 8	+ 7	+25	+12	+16	+23	0	+13 ± 6
Epinephrine, intraocular injection. 0.05 ml. of 1/250,000 dilution (0.2 μg.)								
Grafted eye	—46	—34	—40	—46	—51	—42	—52	—44 ± 2
Normal eye	+18	— 4	+ 8	— 6	+16	+10	+ 3	+ 6 ± 4
Body weight change, grams	—10	—20	—14	—14	+ 4	—12	— 4	

Conclusions

1. By correlation with the fall in ascorbic acid level in the adrenal gland, the eosinopenic response in the peripheral blood has been shown to be an accurate and satisfactory index of the release of cortical steroids from the adrenal, although there is no evidence as yet that the initial level bears any constant relation to the functional capacity of the gland.

2. The initial rise in eosinophiles following either the administration of exogenous epinephrine or the release of endogenous epinephrine is dependent on the contraction of the spleen.

3. A slight but consistent eosinopenic response in adrenalectomized rats can be abolished by concomitant castration. This suggests either a slight overlap in the action of the gonadal and adrenal steroids, or the presence of functioning adrenal cortical cell rests in the gonads.

4. The mechanism of control of the secretion of the adrenal cortex, through the release of adrenocorticotrophic hormone from the pituitary, apparently consists of two phases which may be independent or sequential, depending on the conditions of stress encountered by the organism. These phases have been labelled *autonomic* and *metabolic*—terms which seem descriptive of their physiological characteristics. The first or autonomic phase depends on the reflex secretion of epinephrine which directly activates the anterior pituitary, while the second or metabolic phase is based upon the rate of utilization of adrenal cortical hormones within the organism. This concept has been illustrated in the diagram of Figure 11.

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NEWS AND COMMENT

In this current number of *The Journal,* which begins the second volume since the resignation of Dr. George H. Smith, the Editors wish to try to express to Dr. Smith their deep feeling of gratitude for his years of faithful, devoted service as Editor-in-Chief. In his honor this issue includes a four-color reproduction* of the portrait painted by Deane Keller, and presented to the University by a group of Dr. Smith's friends. The portrait now hangs in the seminar room of Brady three.

It seems appropriate to include here a few lines of appreciation, as expressed by Dr. Samuel C. Harvey at the time of the acceptance of Dr. Smith's resignation, March 22, 1949.

"The close of this academic year will mark the completion of the twenty-first volume of *The Yale Journal of Biology and Medicine.* Unfortunately, it likewise entails the resignation of its editor, Professor George H. Smith, the founder of it and to whom and him alone is due its remarkable success as a medium of scientific communication and as an unofficial organ of the School of Medicine of Yale University.

"Started by him in 1928 without material support from the University or otherwise, as a personal venture he has managed to maintain it through most difficult times on a sound financial basis, at the same time ensuring its quality both in format and content. A measure of his accomplishment is the fact that it rates today and has done so for many years as one of the indispensable journals in any medical library of consequence, both at home and abroad. Many articles of sufficient importance so that they are frequently referred to in the medical literature have appeared in it, and it has served as a medium but not exclusively so for the prompt and comprehensive publication of contributions from the School of Medicine. Over a wide area, it has come to represent most favorably medicine at Yale—in many instances where the School is otherwise unknown.

"During all this time, save in the war years, there has been associated with Professor Smith in the editorial work of *The Journal* a board of student editors who in this association have acquired from him a knowledge of the preparation and editing of articles that has been of the greatest value to them. Even more important has been the intimate personal association with one who, by reason of his mature wisdom and sympathetic understanding, has served to bring to them a high sense of values.

"For these reasons, if for no other, it is inconceivable that *The Journal* should be allowed to lapse, and the burden of the continuance of it, up to now resting solely upon Professor Smith, must be transferred to others for no one person can replace him. It is hoped that his wisdom and moral support will long be available to those who undertake this task."

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* Copies suitable for framing may be obtained from the Managing Editor for seventy-five cents each.

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BOOK REVIEWS

PHYSIOLOGY OF THE NERVOUS SYSTEM. By John Farquhar Fulton. New York, Oxford University Press, 3rd edition, 1949. 667 pp. $10.00.

It would be presumptuous to review this third edition of Fulton's *Physiology of the Nervous System* if it were not for the fact that this is not only a superior book, but also an exceedingly useful one. The form of the book and the exposition of the material are so excellent that re-affirmation of its value needs doing. It comes very near to being a bible for students of the nervous system. It should be read and reread by psychiatrists, neurologists, neurophysiologists, and neuro-anatomists. It is one of the best expositions available on the structure and function of the nervous system and has sufficient clinical material to give significance to the factual information.

Of the twenty-six chapters in the book, a good half have been largely rewritten and brought up to date. To call attention in detail to the more recent developments included would be a work of supererogation. The book must be read, not epitomized. It might be well to call attention, however, to the enlarged section on synaptic transmission and to the added information which has been accumulated through study of cortical mechanisms and their relation to subcortical structures. The chapters on the frontal cortex have been largely rewritten, with particular reference to the relation of this part of the nervous system to human behavior as determined by experimental studies and the clinical results of lobotomy. These chapters are particularly valuable because they present a sane evaluation of the great mass of clinical data resulting from interference of frontal lobe structures and their relationship to other parts of the nervous system. This new departure in the study of the relationship between the nervous system and human behavior is one of the most exciting developments in modern medicine. It is well to note that great impetus was given this procedure as a result of work carried out by Jacobsen in Dr. Fulton's laboratory. No better evaluation of the results so far obtained is available. It provides an excellent foundation for all future investigators in this field.

Valuable as is the text, not the least of its many virtues is the 74-page bibliography. In it are to be found most of the references to major work in the nervous system, thus enabling the student to penetrate the maze of references with assurance. The illustrations are uniformly excellent, particularly because of their clarity. This third edition is not just a reprint, but a thoroughgoing and up-to-date revision of one of the best books in its field.

H. S. BURR.

BLOOD CLOTTING AND ALLIED PROBLEMS. By J. E. Flynn, Editor. New York, Josiah Macy, Jr. Foundation, 1949. 231 pp. $2.25.

The transactions of the Second Conference on Blood Clotting and Allied Problems afford an excellent example of how the current and growing practice of assembling a small number of outstanding investigators in various fields to discuss topics of common interest can facilitate the exchange of information, clarify areas of confusion, and set the stage for rapid advances in knowledge.

The transactions consist of 57 pages of formal presentation of papers by nine authors or teams, and 107 pages of stimulating discussion by nineteen participants in the conference. Topics dealt with formally include a throm-

boplastin inhibitor, mechanism of clot formation, zeta-potential, the conversion of prothrombin to thrombin, the role of platelets, and a synthetic anticoagulant. In addition, there is a special discussion of the general problem of standardization of thromboplastin.

Those with a special interest in blood clotting from the clinical or laboratory standpoints can hardly afford to be unacquainted with the material in this volume. Others will find a wealth of information and much stimulating discussion. Some of the practical wisdom will remain inaccessible to those who do not read every page since there is no index. It is likely that the possibility of indexing such a volume was considered and discarded by the editor. We will be interested to see whether it may not be felt worthwhile in future reports.

V. C. VAUGHN, III.

THE PHYSIOLOGY OF THOUGHT. By Harold Bailey. New York, William-Frederick Press, 1949. 314 pp. $3.75.

The only excuse for reviewing this book in a scientific journal is the hope that someone may be saved from wasting his time in reading it. This book, in spite of the title, is a treatment neither of the physiology of thinking nor of the psychology of thought but a garbled conglomeration of the author's misconceptions in both fields. None of the anecdotes supporting what is claimed as fact is corroborated by either experimental conclusions or documentary evidence.

With a peculiar juxtaposition of vitalism and positivism, the author attributes to certain rather ill-defined cortical cells the function of thought. Activity in these cells is thinking; exercise of them is able to increase capacity to think; inheritance of fewer or less vigorous cells results in feeble-mindedness; disuse leads to atrophy and slow wittedness; recruitment of a majority of cells is concentration, but of a minority is subconscious thought. Habits are thought cells which have been specifically trained, while instincts have been specifically inherited.

The author also deals with dreams, perceptions, illusions, hallucinations, emotions, pain, fatigue, and neurasthenia as particular properties of the thought cells. His naive treatment of these phenomena demonstrates that he is unfamiliar with the critical issues involved in their discussion and his interpretation of them certainly does violation to the few writers he lists in his bibliography.

This book is not only anachronistic, but also inaccurate. The confused logical positivism certainly defeats the alleged purpose of the book—"to provide a popular presentation" of the nature of thought.

H. ENGER ROSVOLD

ORGANIZATION OF BEHAVIOR. By D. O. Hebb. New York, John Wiley & Sons, Inc., 1949. 335 pp. $4.00.

The author has presented a theory of behavior in which he attempts to explain the prevailing problems in the field of psychology. In so doing he has sought after a common ground with anatomy, physiology, and neurology in order that the work of these allied disciplines might be brought to bear directly on problems of behavior.

Basic to the theory is the idea that an often repeated specific stimulation will give rise to the slow development of a diffuse "structure" comprising cells in the cortex and diencephalon. This structure labeled a "cell-assembly"

is conceived of as capable of acting briefly as a closed system which may facilitate the action of other such cell-assemblies and which also has specific motor facilitation. A series of cell-assembly actions constitutes a "phase sequence"; this is the thought process.

The author assumes one fundamental neurophysiological postulate upon which his entire theory seems to rest: "When an axon of Cell A is near enough to excite Cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of firing B, is increased." This neurophysiological expression of the Law of Contiguity permits Dr. Hebb to analyze perception and the development of perceptual learning in terms of the growth and development of, first, cell structure, then cell-assembly, and finally phase sequences. Calling upon conclusions recently reported by Senden regarding the restoration of vision in the congenitally blind, the author makes a strong case for his concept of a learning process in perception. Such a viewpoint takes exception to the prevailing Gestalt theories of perception which have not yet adequately dealt with such data.

Psychophysiological meaning is given to such currently used psychological terms as "set," "attention," "expectancy," "similarity," "generalization," and "abstraction." For example, the central facilitation from a preceding cell-assembly action or by a sensory event or by both of these on another cell-assembly process is considered to be the prototype of "attention."

Motivation, emotion, learning, and intelligence are all dealt with in a painstaking manner. The application of the theory to problems in these areas is certain to be highly provocative to all researchers interested in correlating behavior with physiological function. Rationale for current physical therapies, shock treatment, and frontal lobotomy is offered. Dr. Hebb proposes that if emotional disturbance consists of a disorganized firing of cerebral neurons, then any intervention in the nervous system which would make for the establishment of a hypersynchrony of firing would decrease that emotional disturbance. He suggests that a direct relationship between degree of hypersynchrony as revealed by the electro-encephalogram and reduction of emotional disfunction as observed by the clinician can be established.

Although a major contribution of this monograph includes the reassessment of behavior theory in light of recent investigation in the fields of neurophysiology, it would seem that too little of the theory is firmly grounded in experimental evidence. Considerable weight is placed upon relationships between psychological and physiological mechanisms which may exist only in a spurious fashion. The value of a theory, however, is measured by the amount of useful experimentation it provokes. The challenges to psychologists made by Dr. Hebb, asking them to reconsider many issues upon which present differences regarding psychological theory are based, will undoubtedly stimulate much research. The text contains a fertile environment of postulates which are very amenable to examination in the laboratory.

SYLVAN J. KAPLAN